Molecular ecology and toxicity of *Alexandrium pacificum* in Australian waters

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This thesis has been submitted as the partial fulfillment of the requirements for the degree of Doctor of Philosophy

Certificate of Original Authorship

I, Abanti Barua declare that this thesis, is submitted in fulfilment of the requirements for the award of PhD, in the School of Life Sciences/ Faculty of Science at the University of Technology Sydney.

This thesis is wholly my own work unless otherwise reference or acknowledged. In addition, I certify that all information sources and literature used are indicated in the thesis.

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Thesis Abstract

Species of the genus *Alexandrium* are one of the most studied dinoflagellates due to their production of the neurotoxins, Paralytic Shellfish Toxins (PSTs). PST-associated Harmful Algal Blooms (HABs) appear to be increasing around the world. The appearance of species of *Alexandrium* is now frequent in coastal waters of Australia, particularly in Tasmania and New South Wales. The East Australian Current (EAC) flows southward along the coasts of eastern Australia and has been reported as a global 'climate change hotspot'. Despite such potent neurotoxin production, the ecology, toxicity and population dynamics of *Alexandrium* species are little known in Australia.

In this thesis, I have investigated the first record of PST above the regulatory limit of 0.8 mg/kg produced by *Alexandrium pacificum* in the commercial aquaculture area of south-eastern Australia. During this unprecedented event, the maximum reported PST concentration in mussel tissue was 7.2 mg/kg STX equivalent. A comparative differential gene expression study was conducted to understand the gene regulation of PST related genes in *Alexandrium pacificum*. In this study, experiments were performed in the presence and absence of the copepodamide-synthesizing copepod *Parvocalanus crassirostris*. Using Nanostring gene technology, results identified the up-regulation of the key PST-related gene *sxtA,* in particular, one paralogue each of domains of *sxtA1* and *sxtA4*. An increased rate of PST production in the two PST-producing strains in the presence of copepods was identified, however it did not influence gene related transcript abundance. This indicated that post-transcriptional regulation processes may be important in regulating PST production in *Alexandrium pacificum*. In this thesis, the population structure of *Alexandrium pacificum* was examined in different Australia boundaries currents – the East Australian Current (EAC) and the Leeuwin Current (LC). This study was conducted using Single Nucleotide Polymorphisms (SNPs) as genetic markers and represents the first time the population structure of a phytoplankton species has been examined in Australian waters. Strains from South Australia and Western Australia clustered as a group, and were separated from the strains isolated from the EAC region, indicating the presence of genetic isolation of *A. pacificum* strains in Australian waters. It suggests that *A. pacificum* is more likely to represent a long resident population, and it is not a recent bioinvasion in Western Australian waters.

The results identified during this study significantly advance the understanding of *Alexandrium,* especially their abundance, diversity, population structure and the regulation of the PST-related genes.

Chapter 1:

General Introduction

1.1 Harmful algal blooms (HABs) and their impact

1.1.1 What are Harmful Algal Blooms?

Harmful Algal Blooms (HABs) are naturally occurring phenomenon that occur when certain microalgal species proliferate to a certain level that is sufficient to disrupt the balance of aquatic ecosystems and services dependent on it, such as the environment itself, aquaculture industries, fisheries and tourism (Gobler 2020; Müller, Mardones & Dorantes-Aranda 2020; Smayda 1997b). HABs can also have significant consequences on human health when seafood products are consumed (Anderson, Cembella & Hallegraeff 2012). In older scientific literature, the term "red tides" was used to describe HABs, but later on, a clear definition of HABs was given by Smayda 1997 (Smayda 1997b), which avoided the ambiguity of the fact that not all harmful algal blooms discolour the water column. Dinoflagellates are considered to constitute the majority of marine HAB forming species. Approximately 196 phytoplankton species are identified as potentially harmful, of which 53% belong to dinoflagellates (www.marinespecies.org/hab/).

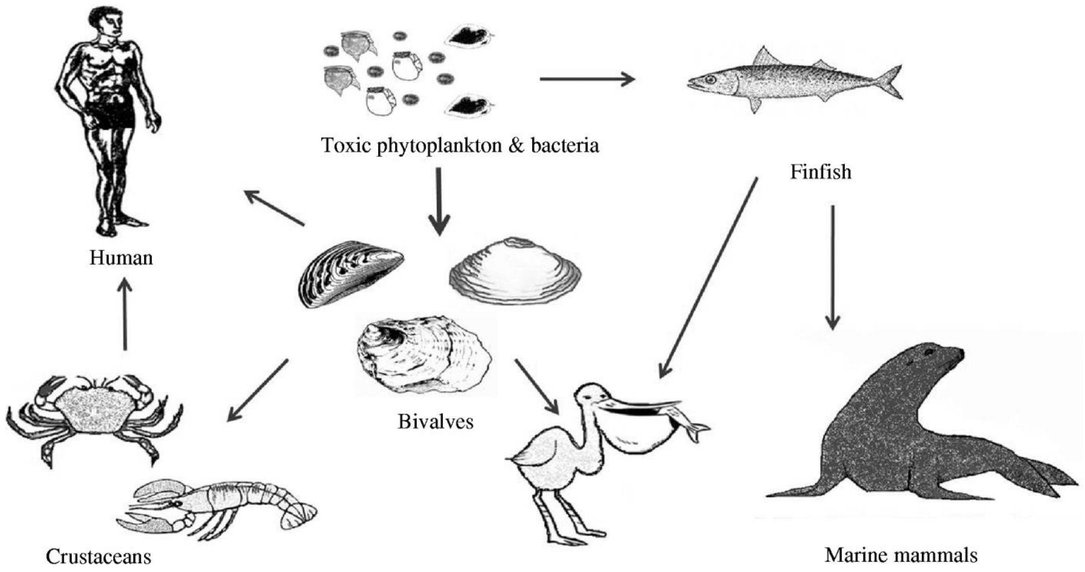


Figure 1: The toxin cycle: diagram illustrating the interrelationships between harmful algae and shellfish, finfish, birds and mammals (James et al., 2010)

Many marine HAB species, especially dinoflagellates and diatoms, are capable of producing potent biotoxins via secondary metabolic pathways (Yasumoto & Murata 1993). These potent chemical compounds can be introduced in the marine food web by bioaccumulation in the tissues of fish, crabs, lobster, abalone or filter-feeding bivalves (shellfish) such as mussels, oysters, scallops and clams (Shumway 1990) (Figure 1). The consumption of such contaminated seafood can transfer these toxins to higher trophic levels affecting birds, mammals and humans.

HAB events have been expanding worldwide in terms of abundance, frequency, intensity, and geographic extent over the past few decades in both marine and freshwaters (Anderson et al. 2008; Glibert et al. 2018; Hallegraeff 1993; Smayda 1990). Climatic drivers of this expansion in HABs are the impacts of ocean warming and marine heatwaves, and non-climatic drivers of this expansion are increased nutrient run-off from land sources, and the discharge of the ballast water from international shipping, which has the potential to distribute species more widely around the world (Glibert et al. 2018; Gobler 2020; Hallegraeff & Bolch 1992; Wells et al. 2015). Intensified HABs in marine waters that have been associated with ocean warming have been observed in several mid and higher latitude regions (Gobler 2020; Gobler et al. 2017; Moore, Mantua & Salathe Jr 2011). Ocean warming is directly connected with the migration of the HABs towards the poles and into deeper oceanic rather than coastal waters (Gobler 2020; Gobler et al. 2017; Hallegraeff et al. 2010). Temperature increases appear to be driving the species range of *Alexandrium catenella* further towards the poles (Brosnahan et al. 2020). Climate change also appears to have had a direct impact on the frequent occurrences of HAB related benthic dinoflagellates like *Gambierdiscus* and *Ostreopsis* (Tester, Litaker & Berdalet 2020).

In the past ten years, the world has experienced three particularly large HAB events which have greatly impacted fisheries and aquaculture industries (Trainer et al 2020) (Figure 2). First one was reported along the west coast of North America, which was expanded from central California to at least as far north as British Columbia in the year 2015 (Trainer et al. 2020). During this *Pseudo-nitzschia* generated HAB, the commercial Dungeness crab fishery alone experienced a loss of USD $ 97.5 million revenue than the earlier year (Trainer et al. 2020). Studies identified the responsibility of the 2014-2016 northeast Pacific marine heatwave for such unprecedented extreme proliferation of this chain-forming diatom genus. The continuous disaster (2012-2017) for the seafood industries in Tasmania, caused by *Alexandrium catenella* (*Alexandrium tamarense* group I) has been considered as a second extreme case. The third expansion was observed in the Chilean coastal waters in 2016, known as "Godzilla-Red tide" which occurred due to the combination of *Pseudochattonella cf. verruculosa* blooms to the south and *A. catenella* to the north oceanic coast of Chile (Clément et al. 2016; Trainer et al. 2020). This event is considered as world's biggest fish farm mortality, and resulted in the loss of ~USD$ 800 million worth of stock (Clément et al. 2016).

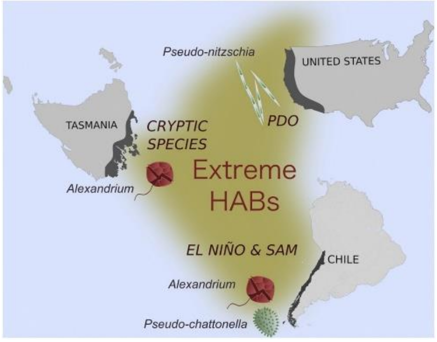


Figure 2. Three extreme HABs (Trainer et al. 2020)

Recently, it has been observed that HAB-related microalgal species composition is shifting. A significant change in the composition of microalgal species has been reported in Korean waters, where the dominancy has shifted from diatoms to dinoflagellates after 1980s (Sakamoto et al. 2020). Dinoflagellates have an efficient inorganic carbon (or CO_2) concentrating mechanisms which help them to grow in high CO_2 conditions, which means increased CO_2 levels may help their growth (Brandenburg, Velthuis & Van de Waal 2019; Gobler 2020; Reinfelder 2011). The competition between microalgal species has changed because of ocean warming and increased CO_2, which may have significantly contributed to the expansion of HAB species (Barton et al. 2020).

The advancement in 'omics techniques is now helping us to understand HAB dynamics, the underlying genetics of toxin biosynthesis, the relationship between climate change and HABs (Hennon & Dyhrman 2020). In addition, these novel molecular technologies can assist us in determining how HABs will change under future climate conditions, and have allowed us to develop early detection systems for HABs to protect human health.

1.1.2 Why do certain HAB species produce toxins?

Compared to the extensive study on the causes and consequences of HABs and their economic and ecological impacts, the role of toxin production for the producing organisms is relatively little known. Several primary mechanisms have been suggested as the major role of biotoxins.

- **Defence against grazers/ copepods**

The biotoxins produced by certain dinoflagellates can provide a chemical defence to deter certain grazers. It has been shown that in the presence of copepods, the production of bioactive compounds like saxitoxins, spirolides or gymnodimimes increased in *Alexandrium* species ((Bagøien et al. 1996; Selander et al. 2006; Wohlrab, Iversen & John 2010; Yang et al. 2011). A similar activity can be observed in *Karenia brevis.* Brevetoxins produced by this dinoflagellate can affect grazing activity (Hong et al. 2012) (Figure 3). It has been reported that the copepods have potential to the produce chemical compounds known as copepodamide (Selander et al. 2015), which appears to act as a signal for *Alexandrium* species to increase their toxin production. Only a pico or nano molar concentration of these chemicals is sufficient to increase the paralytic shellfish toxins (PSTs) production up to 20-fold in *Alexandrium minutum* (Selander et al. 2015).

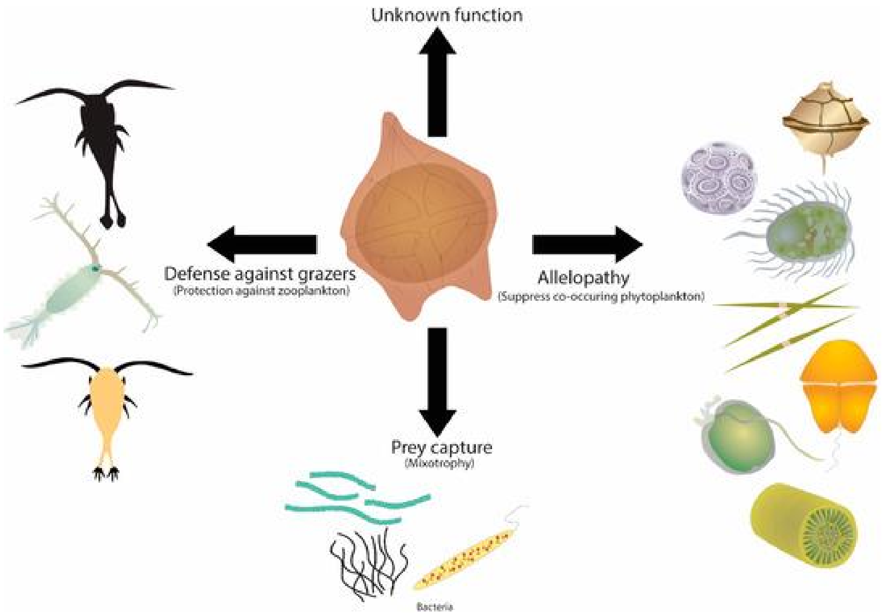


Figure 3: Possible roles of toxins produced by the HAB related dinoflagellates (Verma et al. 2019).

- **Allelopathy:**

HAB species excrete extracellular lytic metabolites, known as allelochemicals, which have the potential to cause the death or the growth suppression of other plankton species (Blossom & Hansen 2020; Tillmann et al. 2008; Tillmann & Hansen 2009; Tillmann & John 2002b; Tillmann et al. 2014). *Alexandrium* species are capable of producing such bioactive compounds that could cause an allelopathic effect on a wide range of organisms including microbial communities, phytoplankton and co-occurring heterotrophic protists (Murray, John & Kremp 2015; Tillmann et al. 2008; Tillmann & Hansen 2009; Tillmann & John 2002b; Tillmann et al. 2014). *A. catenella (A tamarense group 1)* shows allelopathic activity that helps them togrow well in the presence of the heterotrophic predator *Polykrikos kofoidii* (John et al. 2015). Karlotoxins, produced by *Karlodinium veneficum* can interfere in the growth of co-occurring plankton and can immobilize the prey (Waggett, Tester & Place 2008) (Figure 3).

- **Prey Capture:**

The HAB species *Akashiwo sanguinea, Alexandrium tamarense, Gymnodinium catenatum, G. impudicum, Heterocapsa triquetra*, *Karlodinium armiger, K. veneficum, Lingulodinium polyedrum, Prorocentrum donghaiense, P. micans, P. minimum, P. triestinum, Scrippsiella trochoidea* have been reported as mixotrophic, meaning that they are both photosynthetic and can obtain nutrition through the capture and digestion of certain preys (Berge, Hansen & Moestrup 2008; Jeong et al. 2005). It has been proposed that HAB toxins may assist some species in their capture of certain prey species. The ability to use both phototrophy and phagotrophy could possibly stimulate the growth of these HAB species and therefore assist in HAB initiation and maintenance (Flynn et al. 2019). (Figure 3)

1.1.3 Impacts of HABs

Marine biotoxins produced during HABs can result in seafood poisoning syndromes in humans, and lead to illness and even death in severe cases (Table 1). Aerosolized toxins have also been reported to cause harm to human respiratory systems (Backer et al. 2003). Every year, about 60000 human intoxications occur, with an overall mortality of about 1.5% (Kantiani et al. 2010)

a) HABs can also lead to significant economic losses in fisheries and aquaculture industries worldwide. Aquaculture is now at a significant risk with the increased frequency of HABs throughout the world, as they generally require close monitoring and harvesting closures as preventative measures if HAB species are detected, or they can lead to lost economic opportunities as seafood imports or sales are banned or product recalls are required if biotoxins are detected in seafood. In the United States, ˜ $100 M is lost annually due to HABs (Griffith & Gobler 2020). Large scale deaths of caged fish and shellfish were documented due to HABs (Anderson et al. 2000). A single event in Tasmania led to ~AUD$23 million in costs, due to a large scale HAB of *Alexandrium catenella* in 2012/13 along the east coast of Tasmania (Campbell et al. 2013). This event significantly impacted the market access of mostly export-focused Tasmanian lobster fishery as PST can concentrate in the hepatopancreas of the lobster (Turnbull et al. 2020). Another notable economic loss of ~USD$ 100 M was observed in South Korea resulted by a bloom of *Cochlodinium polykrikoides* (Griffith & Gobler 2020; Kim et al. 2002).

b) HABs can severely affect the tourism industry and related industries that are dependent on tourism. Recurrent HABs of *Karenia brevis* that occurred along the southwest coast of Florida from 2002 to 2018 have caused fishing shutdowns, beachside restaurant closures, and beach closures related to large scale fish deaths (Bechard 2019).

c) HABs and related toxins were identified as a threat for the marine mammals, fish, sea birds, invertebrates, and sea grasses (Landsberg 2002). During the red tide of *Karenia brevis* in the Gulf of Mexico, a 26 feet long whale shark and thousands of fishes were killed (Bechard 2019).

Table 1: Major seafood poisoning caused by different biotoxins from marine algal species and effects on human (Adapted and modified from (Kalaitzis et al. 2010; Visciano et al. 2016; Wang 2008)

Type of poisoning	Toxins	Responsible taxa	Symptomatology on human	Primary vector
Paralytic Shellfish Poisoning (PSP)	Saxitoxin (STX) and its analogues	*Alexandrium* spp., *Gymnodinium* spp., *Pyrodinium* spp.	• Gastrointestinal symptoms • Paralysis of the respiratory tract • Recovery or death	Shellfish
Neurotoxic Shellfish Poisoning (NSP)	Brevetoxins (BTXs) and its analogues	*Karenia brevis,* *Chatonella marina,* *C. antiqua,* *Fibrocapsa japonica,* *Heterosigma akashiwo*	• Gastrointestinal symptoms • Respiratory problems • Recovery or death	Shellfish
Ciguatera Fish Poisoning (CFP)	Ciguatoxins (CTXs)	*Gambierdiscus* spp.	• Gastrointestinal symptoms • Cardiovascular or neurological problems	Coral reef fish
	Maitotoxin (MTX)	*Gambierdiscus* spp.		
Diarrhetic Shellfish Poisoning (DSP)	Okadaic acid (OA) Dinophysis toxins (DTXs)	*Dinophysis* spp., *Prorocentrum* spp.	• Gastrointestinal symptoms • Recovery in 3 days	Shellfish
	Pectenotoxin	*Dinophysis* spp.		
	Yessotoxins (YTX)	*Protoceratium reticulatum,*		

		Lingulodinium polydrum and Gonyaulax spinifera		
Azaspiracid Shellfish Poisoning (AZP) (In several studies, this poisoning was referred as DSP)	Azaspiracids (AZAs)	*Protoperidinium Crassipes, Azadinium spinosum*	• Gastrointestinal symptoms	Shellfish
Amnesic Shellfish poisoning (ASP)	Domoic acid (DA)	*Pseudonitzschia* spp., *Nitzschia* spp.,	• Gastrointestinal and neurological symptoms • Cardiac or respiratory problem • Recovery or death	Shellfish
Palytoxin Poisoning	Palytoxins (PLTXs)	*Ostreopsis* spp. *Palythoa* spp.	• Gastrointestinal symptoms • Muscle and cutaneous problems	Shellfish

1.2 Paralytic Shellfish Toxins and Poisoning

1.2.1 Paralytic Shellfish Toxin (PST)

A group of naturally occurring alkaloid type neurotoxins that are commonly known as paralytic shellfish toxins (PSTs), are the main cause of paralytic shellfish poisoning (PSP). Saxitoxin, the most studied PST since its discovery, and its 57 analogs, are the member of this neurotoxin group (Anderson, Glibert & Burkholder 2002; Kellmann et al. 2010; Sellner, Doucette & Kirkpatrick 2003; Wiese et al. 2010). In the marine environment, saxitoxin can be produced by eukaryotic dinoflagellates, whereas cyanobacteria can produce saxitoxin in fresh water(Wiese et al. 2010). STX is too toxic to humans that only 1 mg of the toxin is enough for a fatality (Wiese et al. 2010). STX has been enlisted as “toxin as weapon” for bioterrorism (Anderson 2012). In 1957, the pure STX was first isolated from Alaska butter clam, *Saxidomus gigangteus* (Schantz et al. 1975). PSTs can directly affect a wide variety of marine organisms including mollusks, crustaceans, starfish, octopus, fish, turtles, marine mammals and birds, and human either by direct accumulation or vector transmission (Murray, John & Kremp 2015).

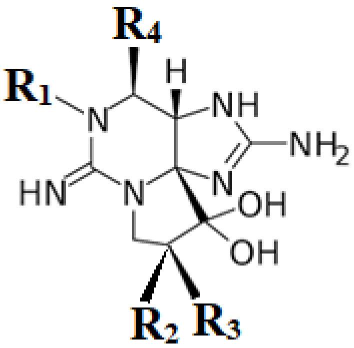


Figure 4: Basic Molecular structure of saxitoxin (Modified and adapted from (Wang 2008)

The basic structure of STX is trialkyl tetrahydropurine, with an NH2 group at the positions of 2 and 8 of the purine (Schantz et al. 1975) (Figure 4). Derivatives of STX are obtained depending on the variations in functional groups at four defined positions around the ring (Table 2). Carbamate toxin, N -sulfocarbamoyl toxin, dicarbamoyl toxin and deoxycarbamoyl toxin are the four divisions of the saxitoxin group (Wang 2008). The most highly toxic group is the carbamate group, and within this group saxitoxin (STX) is the most toxic, followed by neosaxitoxin and gonyautoxins 1 and 3 (Llewellyn 2006). The sulfocarbamoyl saxitoxin group is less toxic compared to other groups (Wang 2008).

Table 2: Saxitoxin derivatives produced by marine dinoflagellates (modified and adapted from (Wang 2008)).

	Toxin	**R1**	**R2**	**R3**	**R4**	**Sub group name (if present)**
Carbamate	STX	H	H	H	OCONH2	
	Neo STX	OH	H	H	OCONH2	
	GTX1	OH	OSO3^{-}	H	OCONH2	Monosulfated
	GTX2	H	OSO3^{-}	H	OCONH2	Monosulfated
	GTX3	H	H	OSO3^{-}	OCONH2	Monosulfated
	GTX4	OH	H	OSO3^{-}	OCONH2	Monosulfated
N-sulfocarbamoyl	GTX5(B1)	H	H	H	OCONHSO3^{-}	Monosulfated
	GTX6(B2)	OH	H	H	OCONHSO3^{-}	Monosulfated
	C1	H	OSO3^{-}	H	OCONHSO3^{-}	Di-sulfated
	C2	H	H	OSO3-	OCONHSO3^{-}	Di-sulfated
	C3	OH	OSO3^{-}	H	OCONHSO3^{-}	Di-sulfated
	C4	OH	H	OSO3-	OCONHSO3^{-}	Di-sulfated
Decarbamoyl	dcSTX	H	H	H	OH	
	dcNeoSTX	OH	H	H	OH	
	dcGTX1	OH	OSO3-	H	OH	
	dcGTX2	H	OSO3-	H	OH	
	dcGTX3	H	H	OSO3-	OH	
	dcGTX4	OH	H	OSO3-	OH	
Deoxydecarbamoyl	doSTX	H	H	H	H	
	doGTX2	H	H	OSO3-	H	
	doGTX3	H	OSO3-	H	H	

Beside the above mentioned derivatives, some other analogues of saxitoxin have also been reported to be present in *Gymnodinium catenatum* (Wiese et al. 2010).

Like other marine neurotoxins, sodium channels are the primary targets of STX and its derivatives (Cestèle & Catterall 2000). With high affinity, these toxins bind to the voltage-gated sodium channels in nerve and muscle cells and can cause death by respiratory paralysis (Catterall 1985) (Figure 5). Potassium and calcium ion channels can also be a target of these toxins (Su et al. 2004; Wang, Salata & Bennett 2003) with a different mechanism of action (Su et al. 2004; Wang, Salata & Bennett 2003). These toxins block the ion channel and inhibit the rapid and passive diffusion of the ion across the lipid bilayer.

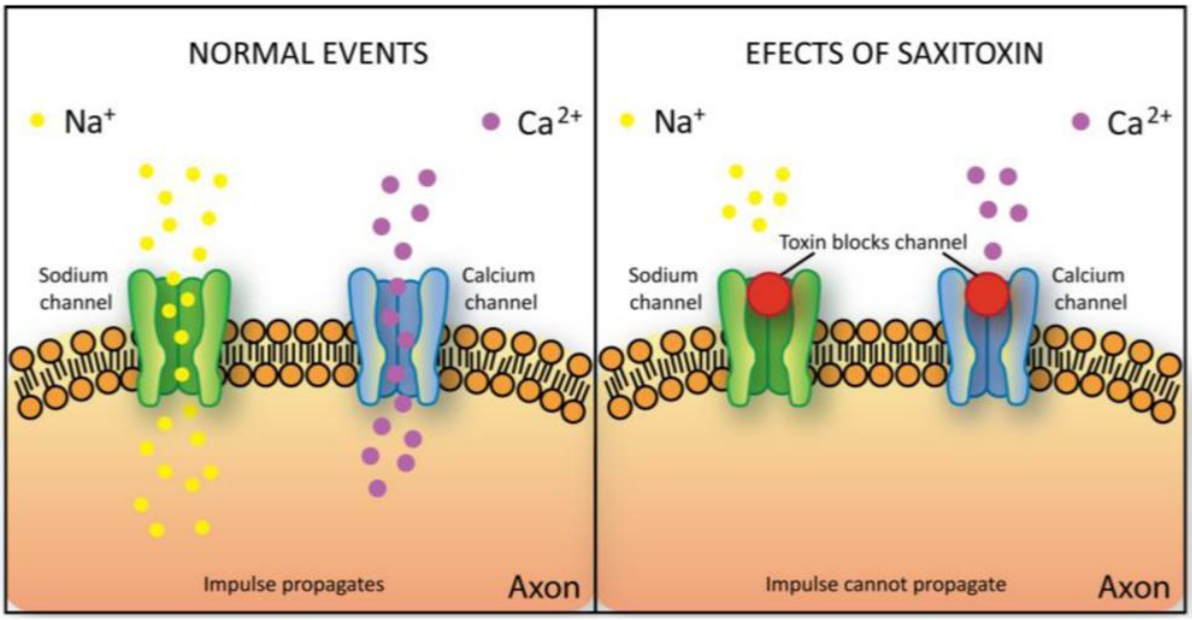


Figure 5: Schematic representation of saxitoxin toxicity mechanism. (Valério, Chaves & Tenreiro 2010)

Different strains of PST producing dinoflagellates produce different variants of saxitoxins (reviewed in (Wiese et al. 2010). The content and profile of the toxin can also be affected by different factors like salinity (Suikkanen et al. 2013), temperature (Anderson et al. 1990) availability of nutrients (Anderson et al. 1990), nutrient supplement (Wang & Hsieh 2002), the presence of grazers (Bergkvist, Selander & Pavia 2008), and intracellular arginine concentration (Anderson et al. 1990).

1.2.2 Paralytic Shellfish Poisoning (PSP)

The consumption of seafood contaminated with PSTs can lead to PSP by interacting with the nervous system (as described above) (Figure 6). Symptoms included paraesthesia and numbness, muscular weakness, the sensation of lightness and floating, ataxia, motor incoordination, drowsiness, incoherence and progressively decreasing ventilation efficiency and can lead to respiratory failure and death in the case of severe intoxication (Deeds et al. 2008). Figure 6 has outlined how PSP can be developed from *Alexandrium.*

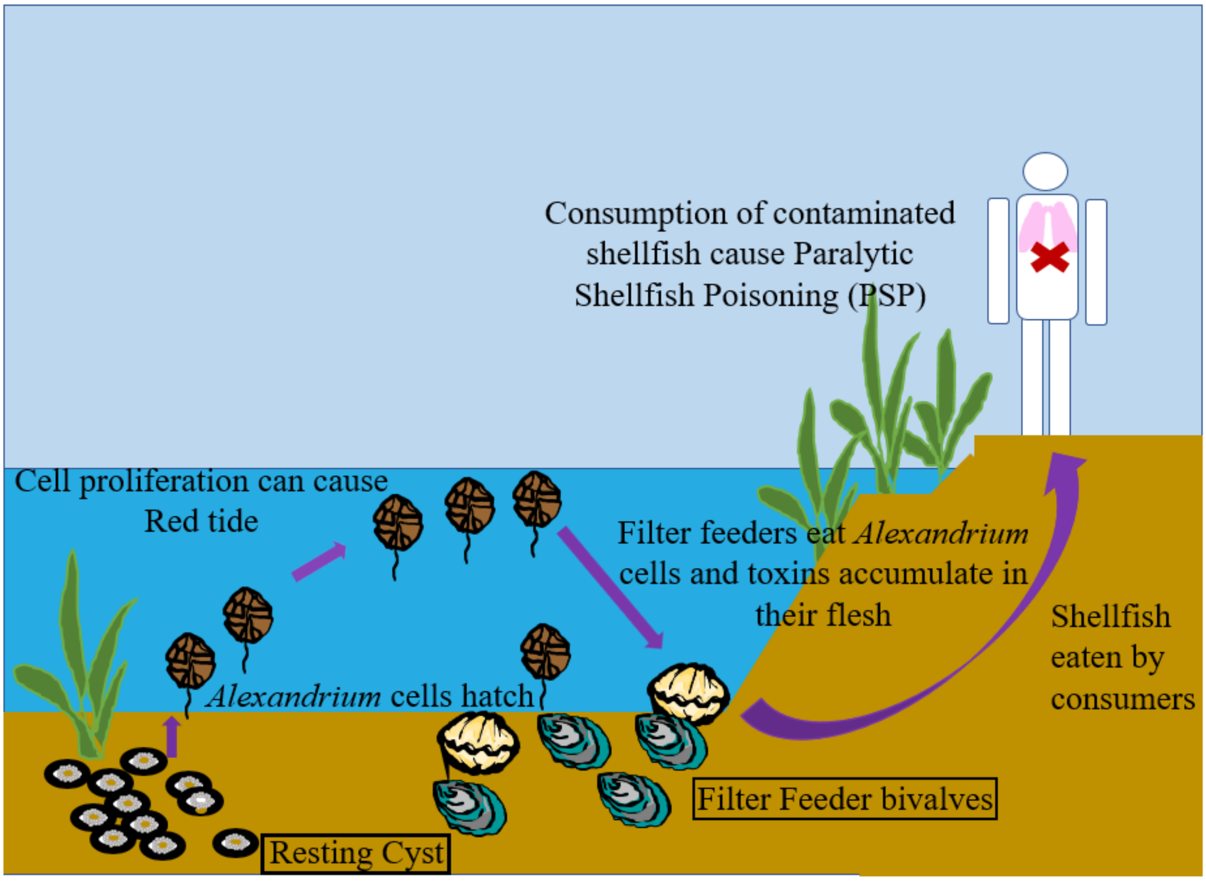


Figure 6: The process of PST accumulation in shellfish and human PST poisoning.

1.3 PSP and PST events

1.3.1 PSP and PST events worldwide

Paralytic shellfish poisoning (PSP) is a fatal human illness which is becoming more widespread in recent years (Figure 7).

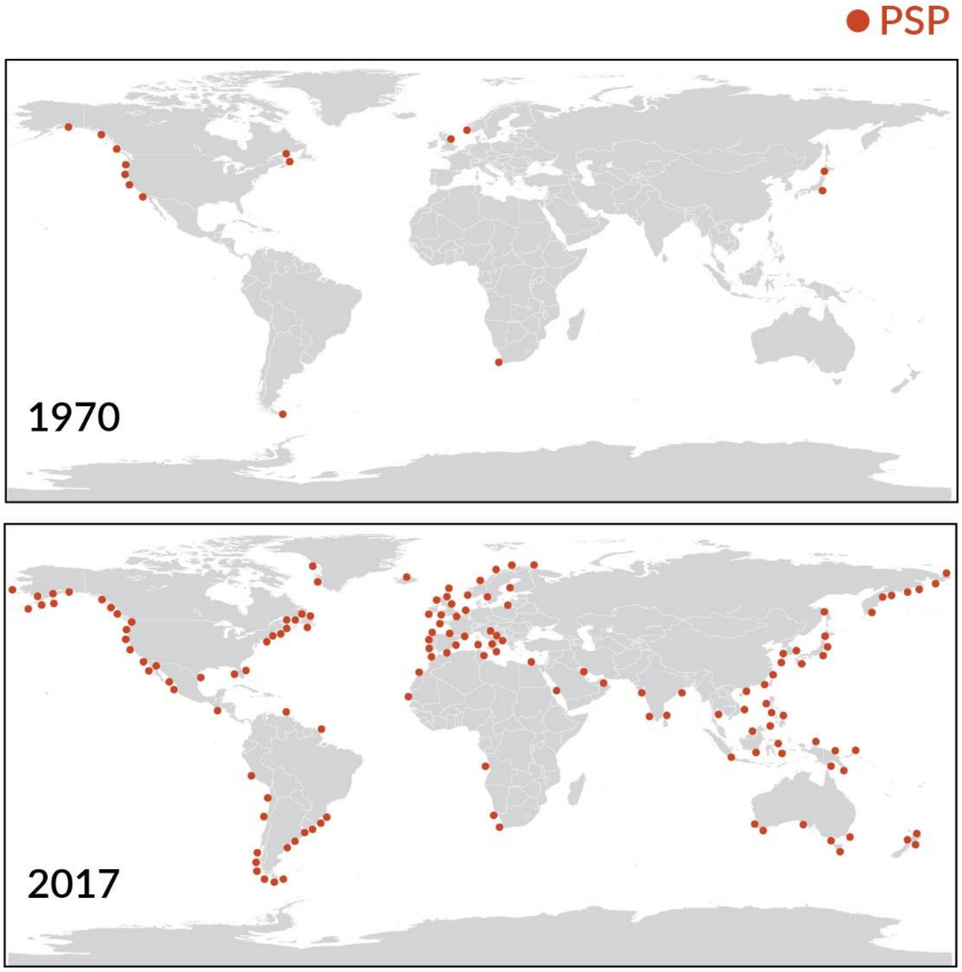


Figure 7: Comparative representation of PSP distribution worldwide from 1970 to 2017 (US national Office for Harmful Algal blooms https://hab.whoi.edu/maps/regions-world-distribution/)

PSTs have a significant impact on human health and wellbeing. In the southeast Asian region, HABs are most common in the Philippines and Malaysia. The intensity and frequency of PSTs have been increased in past 30 years (Yñiguez & Ottong 2020). From 1983 to 2005, the Philippines recorded 2161 cases of STX poisoning with 123 fatalities (Asakawa et al. 2015).

Alexandrium pacificum has been reported to form toxic blooms on the French Mediterranean coast causing significant economic loss due to shellfish closures periodically from 2001 to 2007 (Laabir et al. 2007). In July 2020, a severe PST event was recorded in Alaska with the concentration of toxin identified as 11,200 µg in 100 gm mussel flesh. It was around 140 times higher than the regulatory limit 80 µg per 100 gm of meat according to the U.S. food and Drug Administration guideline (https://www.arctictoday.com/alaskas-first-shellfish-toxin-death-in-10-years-comes-amid-signs-of-spreading-harmful-algal-blooms/).

Furthermore, PSTs events have been identified as very common event in Latin America. PST events are frequently documented events in the Mexican Pacific, the Gulf of Mexico, Brazilian waters, Southern and Northern Chile regularly (Band-Schmidt et al. 2010; Band-Schmidt et al. 2019; Müller, Mardones & Dorantes-Aranda 2020). Large scale sea turtle deaths have been reported along the Pacific coastline of El Salvador in the period of 2013 to 2017 which was caused by PSTs (Amaya et al. 2018). With the repeated report of increased frequency and intensity of PSTs, the reports on the expansion of these species in new geographical area has also been reported. Recently, *A. tamiyavanichii* was identified in central Mexican Pacific which was not known in that area (Band-Schmidt et al. 2019).

1.3.2 PSP and PSTs in Australia

In Australia, *Gymnodinium catenatum* and several species of *Alexandrium* are two main genera of dinoflagellates that have been implicated for all of the PST events and PSP cases (Hallegraeff et al. 2020) (Figure 8). *Alexandrium* is the most common genus in Australia, and the presence of numerous species in Australian coastal waters has been reported. These are: *Alexandrium affine, A. catenella* Group IV ribotype (=*Alexandrium pacificum*), *A. fraterculus, A. margalefi, A. minutum, A. ostenfeldii, A. pseudogonyaulax, A. tamarense* Group V ribotype (= *A. australiense*), *A. fundyense,* and *A. diversaporum* (Ajani et al. 2013; Farrell et al. 2013; Hallegraeff et al. 1991; Hallegraeff et al. 2010; Murray et al. 2014; Murray et al. 2012). Among them, *Alexandrium pacificum, A. minutum, A. fundyense, A. ostenfeldii* have been reported as potential toxin producers (Table 4). The major PST events have been documented in Table 3.

PSP was unknown in Australia until 1980, except for a report in the Australian medical literature in February 1935 where they observed typical PSP symptoms that were produced by wild mussels collected from Batemans Bay, NSW (Le Messurier 1935). The causative organism was identified as *Alexandrium pacificum* (Hallegraeff et al. 1991; Hallegraeff et al. 2020) which had also been reported causing significant toxicity in wild mussels in Port Philip Bay, Victoria in 1986 (Hallegraeff 1992). This latter event was the first report of the presence of *Alexandrium* in Australian coastal waters. On the same year, *A. minutum* was observed to form "red tide" in the Port River in South Australia (Hallegraeff 1992). Since then, this species was observed to cause red tide bloom (up to 300,000 cells per litre) in several years, from September to November (Cannon 1990; Hallegraeff 1992; Hallegraeff et al. 2020). The presence of *A. minutum* has been recorded in the Swan River, Western Australia, since 1983 (Dias et al. 2015). Recently, the presence of *A. pacificum* has also been recorded from the estuaries of Western Australia (Dias et al. 2015). No *Alexandrium* bloom have been found from this part of Australia.

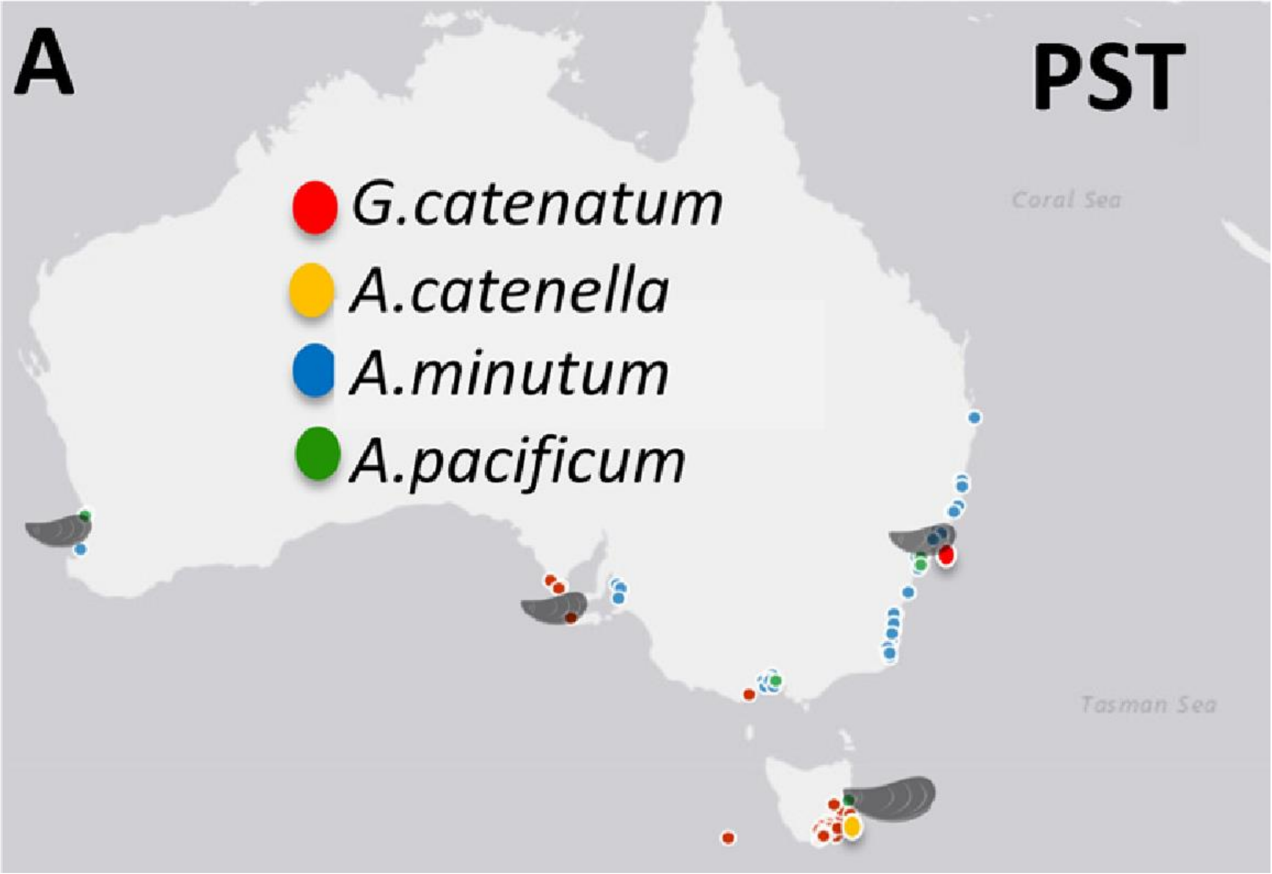


Figure 8: PST events in Australian Coastal water (Adopted and modified from (Hallegraeff et al. 2020).

Two Australian states, New South Wales (NSW) and Tasmania, have been most affected by PST events. These states are located adjacent to the East Australian Current system, a climate change "hot spot'. Since 1985, *Gymnodinium catenatum* has caused several blooms in Tasmanian coastal waster with

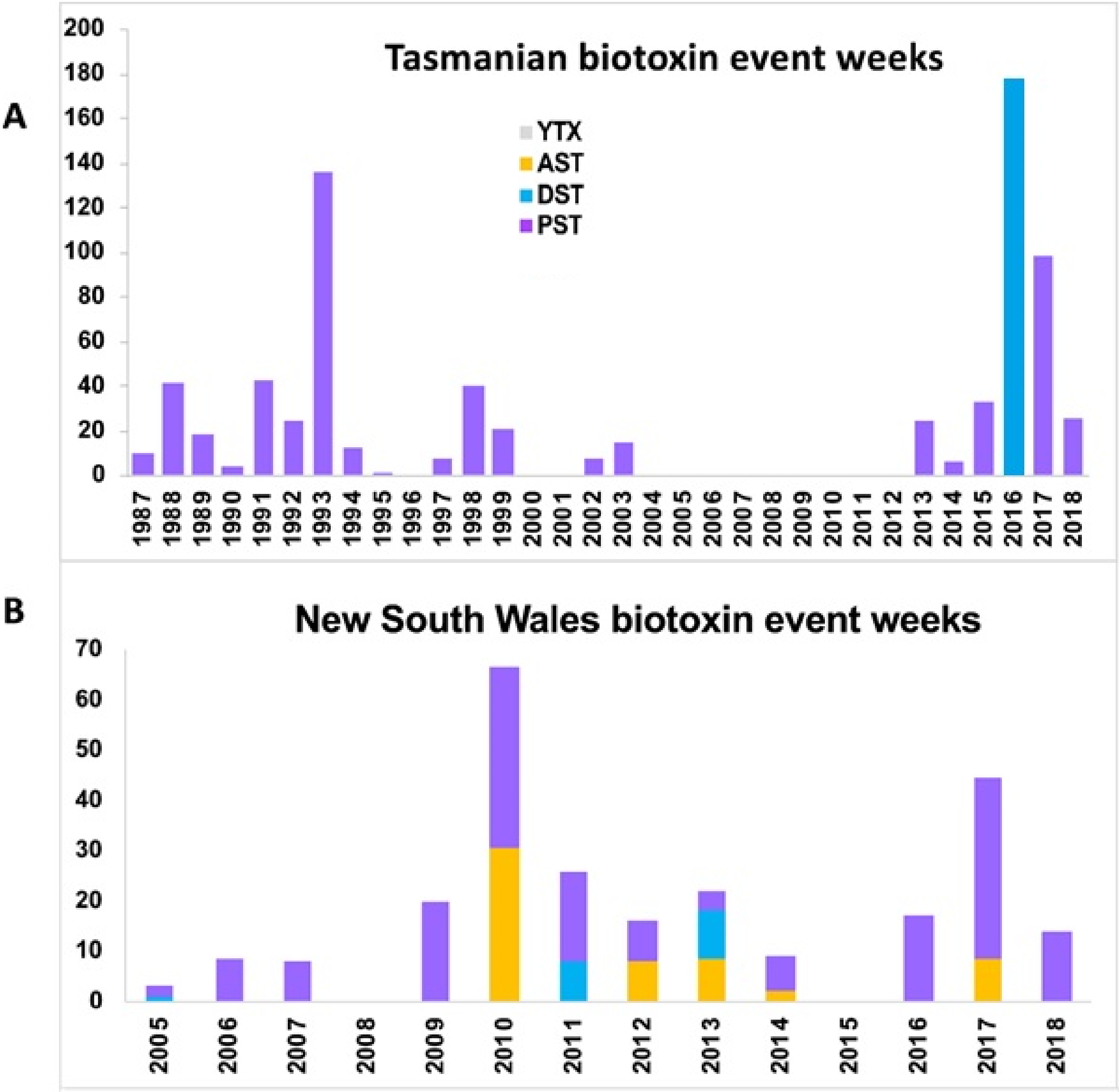


Figure 9: Shellfish farm closure events in A. Tasmania (1987-2018), B. New South Wales (2005- 2018)

a temporary farming closure for up to 6 months in 1985. The most recent and most significant PST event in Australia occurred in 2012, whereby a shipment of blue mussels (*Mytilus galloprovincialis*) that was sourced from the east coast of Tasmania (Ajani et al., 2016 and references therein) was found to be toxic by Japanese authorities. This toxic event cost the Australian industry a total economic loss of ~$23M. It was later identified that the mussels had been contaminated by PSTs from a bloom of *A. catenella (A. tamarense* Group 1). Significant levels of PST were also recorded in scallops, clams, and rock lobsters with a resulting six months harvest closure along 350 km of the Tasmanian coastline (Campbell et al. 2013). Though in this coastal water *A. catenella* was utterly unknown in Australian water before that event, but it is now being considered as annual event in Tasmania (Hallegraeff et al. 2020) (Figure 9). In Tasmania, the severe toxicity was observed in the year 2017 when the toxin detection was confirmed as 150 mg/Kg PST in mussels (Condie, Oliver & Hallegraeff 2019). During this period (2012-2017), four nonfatal human poisonings were also recorded (Hallegraeff et al. 2020).

NSW is considered as another significantly affected Australian coastal area. The PST events in this area are described the next section.

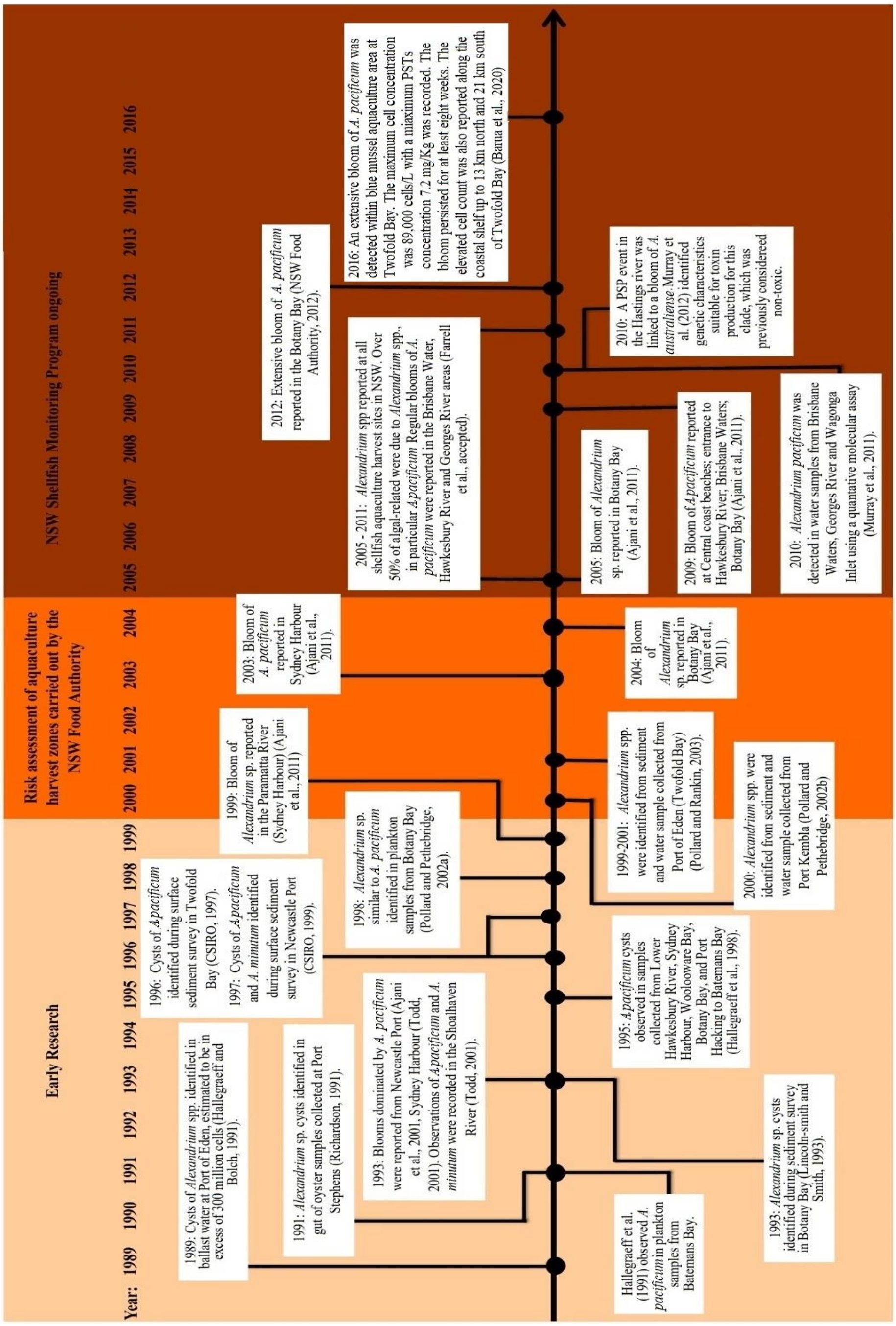
Table 3: Major PST events and toxicity level along Australian coast

Responsible species	Location	Year	Highest recorded toxicity	References
A. pacificum	Port Philip Bay, Victoria	1986	4.8 mg/kg	(Hallegraeff et al. 1991)
A. minutum	Port River, SA	1988	up to 27 mg/kg	(Cannon 1990)
A. pacificum	Sydney Harbour	1993	3 mg/kg	(Bolch & de Salas 2007)
A. pacificum	Twofold Bay	2016	7.2 mg/kg	(Barua et al. 2020)
A. catenella	East coast of Tasmania	2017	150 mg/kg	(Condie, Oliver & Hallegraeff 2019)

Gymnodinium catenatum	Huon River and d' Entrecasteaux Channel	1985	200mg STX eq./kg	(Hallegraeff et al. 2012)

1.3.3 PST Events and New South Wales (NSW) Coastal Waters

Aquaculture is considered as one of the most economically significant industries in Australia. The NSW shellfish aquaculture industry alone produces around $AUD 54.5 million during 2017/2018 (NSWDPI. 2019). The Sydney rock oyster (*Saccostrea glomerata*) is the main species grown and harvested and alone produces ~ $AUD 28.5 million per year (Ajani et al. 2013; Farrell, Ajani, et al. 2014).



1989: Cysts of *Alexandrium* spp. identified in ballast water at Port of Eden, estimated to be in excess of 300 million cells (Hallegraeff and Bolch, 1991).

Hallegraeff et al. (1991) observed *A. pacificum* in plankton samples from Batemans Bay.

1991: *Alexandrium* sp. cysts identified in gut of oyster samples collected at Port Stephens (Richardson, 1991).

1993: Blooms dominated by *A. pacificum* were reported from Newcastle Port (Ajani et al., 2001, Sydney Harbour (Todd, 2001). Observations of *A.pacificum* and *A. minutum* were recorded in the Shoalhaven River (Todd, 2001).

1993: *Alexandrium* sp. cysts identified during sediment survey in Botany Bay (Lincoln-smith and Smith, 1993).

1995: *A.pacificum* cysts observed in samples collected from Lower Hawkesbury River, Sydney Harbour, Woolooware Bay, Botany Bay, and Port Hacking to Batemans Bay (Hallegraeff et al., 1998).

1996: Cysts of *A.pacificum* identified during surface sediment survey in Twofold Bay (CSIRO, 1997).

1997: Cysts of *A.pacificum* and *A. minutum* identified during surface sediment survey in Newcastle Port (CSIRO, 1999).

1998: *Alexandrium* sp. similar to *A. pacificum* identified in plankton samples from Botany Bay (Pollard and Pethebridge, 2002a).

1999: Bloom of *Alexandrium* sp. reported in the Paramatta River (Sydney Harbour) (Ajani et al., 2011)

1999-2001: *Alexandrium* spp. were identified from sediment and water sample collected from Port of Eden (Twofold Bay) (Pollard and Rankin, 2003).

2000: *Alexandrium* spp. were identified from sediment and water sample collected from Port Kembla (Pollard and Pethebridge, 2002b)

2003: Bloom of *A. pacificum* reported in Sydney Harbour (Ajani et al., 2011).

2004: Bloom of *Alexandrium* sp. reported in Botany Bay (Ajani et al., 2011).

2005: Bloom of *Alexandrium* sp. reported in Botany Bay (Ajani et al., 2011).

2005 - 2011: *Alexandrium* spp reported at all shellfish aquaculture harvest sites in NSW. Over 50% of algal-related were due to *Alexandrium* spp., in particular *A.pacificum* Regular blooms of *A. pacificum* were reported in the Brisbane Water, Hawkesbury River and Georges River areas (Farrell et al., accepted).

2009: Bloom of *A.pacificum* reported at Central coast beaches; entrance to Hawkesbury River; Brisbane Waters; Botany Bay (Ajani et al., 2011).

2010: *Alexandrium pacificum* was detected in water samples from Brisbane Waters, Georges River and Wagonga Inlet using a quantative molecular assay (Murray et al., 2011).

2010: A PSP event in the Hastings river was linked to a bloom of *A. australiense*. Murray et al. (2012) identified genetic characteristics suitable for toxin production for this clade, which was previously considereed non-toxic.

2012: Extensive bloom of *A. pacificum* reported in the Botany Bay (NSW Food Authority, 2012).

2016: An extensive bloom of *A. pacificum* was detected within blue mussel aquaculture area at Twofold Bay. The maximum cell concentration was 89,000 cells/L with a miaximum PSTs concentration 7.2 mg/Kg was recorded. The bloom persisted for at least eight weeks. The elevated cell count was also reported along the coastal shelf up to 13 km north and 21 km south of Twofold Bay (Barua et al., 2020)

Figure 10: Timeline of early research and current developments in the understanding of distribution and dynamics of *Alexandrium spp* in NSW. (Adapted and modified (Farrell, Ajani, et al. 2014)

However, the aquaculture industry in New South Wales is being threatened by HABs. According to NSW Food Authority, *Alexandrium* spp. and associated PST events are considered to be the major issue for aquaculture zones in NSW (NSWFA. 2017) (Figure 9 B). It has been reported that the maximum concentration of PSTs so far detected in the NSW shellfish harvest is 7.2 mg/kg (Barua et al. 2020). Figure 10 shows the timeline of early research and current developments (1989-2016) in the understanding of the distribution and dynamics of *Alexandrium spp.* in NSW.

To protect these immensely valuable aquaculture industries from microalgal threat, regular monitoring of harmful microalgae in NSW oyster growing estuaries is important. Along the NSW coastline (over 2000 km (28°10'-37° 30' S)), the NSW Food Authority in conjunction with oyster farmers regularly carries out phytoplankton and biotoxin monitoring in 74 harvest areas along the coast (Ajani et al. 2013; Farrell, Brett, et al. 2014) (Figure 11). The NSW Food Authority also regulates a seasonal (June to November) management and monitoring program of wild harvest at 36 beaches (Farrell, Brett, et al. 2014). Figure 11 shows the summary of positive PST detections across NSW estuaries. It has been observed that Twofold bay was the only area where the PST level exceeded the regulatory limit. All other harvest areas along the estuaries did not have any record the PST level higher than 0.66 mg/Kg (NSWFA. 2017). These monitoring programs play a vital role in minimizing the public health risks and ensuring the sustainability of the aquaculture industry. Furthermore, to determine the risk posed by *Alexandrium* species in each harvest zone, regular monitoring of the phytoplankton community and biotoxin have been carried out since 2005, across ~2000 km of NSW coastline (Farrell, Brett, et al. 2014). All other states of Australia have similar monitoring programs to minimize the PSP risk (Farrell et al. 2013).

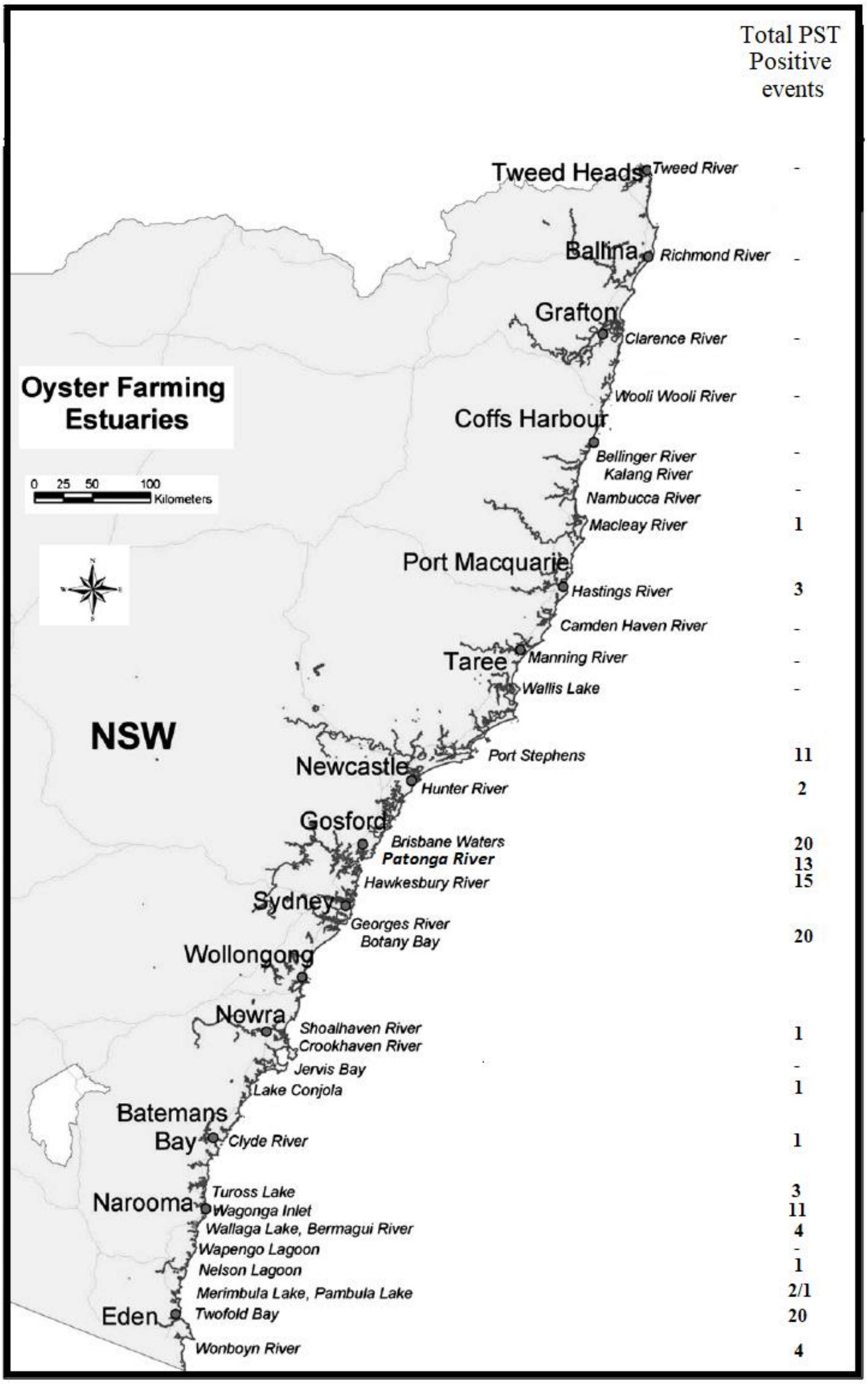


Figure 11: Location of oyster producing estuaries along the coastline of New South Wales, Australia and PST positive events along the estuaries.

1.4 Dinoflagellates related to PSP

A group of marine dinoflagellates consisting of certain species of the genus *Alexandrium* (Anderson, Cembella & Hallegraeff 2012), *Pyrodinium bahamense* (Maclean 1989)(Maclean, 1989), and *Gymnodinium catenatum* (Oshima et al. 1987) are responsible for Paralytic Shellfish Poisoning (PSP) (Figure 12). They produce saxitoxin and its analogous (STX) which is a threat to human health via the consumption of contaminated seafood.

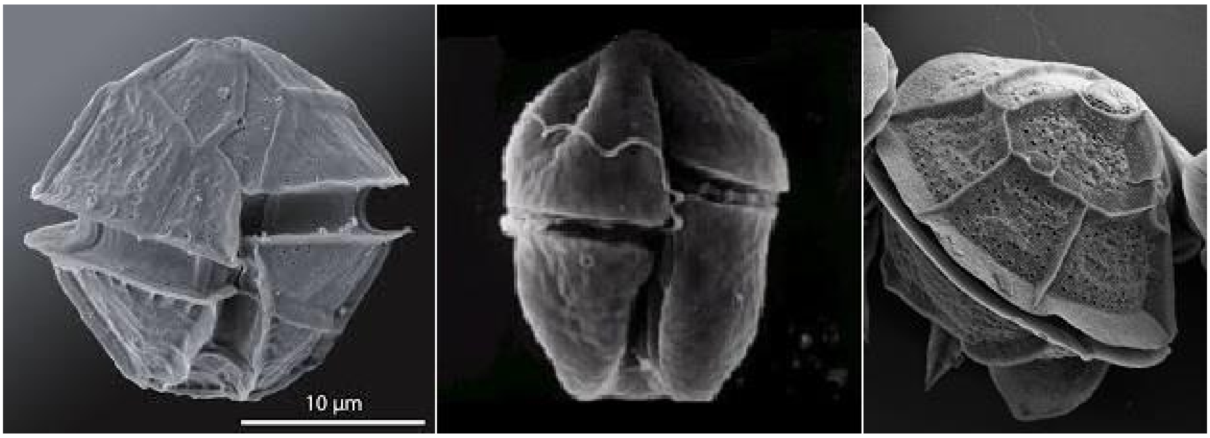


Figure 12: Scanning electron microscopic image of (left to right) *Alexandrium* sp. (Hansen, Daugbjerg & Franco 2003), *Gymnodinium catenatum* (Lundgreen 2018) and *Pyrodinium bahamense* (Banguera-Hinestroza et al. 2016).

1.4.1 Alexandrium

The genus *Alexandrium* consists of some of the most widely studied dinoflagellate species, due to their impacts on humans and ecosystems (Anderson, Cembella & Hallegraeff 2012). For over 30 years, species of *Alexandrium* have been studied extensively for their potential neurotoxin producing capacity (Murray, John & Kremp 2015). The name "*Alexandrium"* was first used by Halim to describe this tiny pigmented algae when he observed a water discoloration (a 'red tide') caused by *Alexandrium minutum* in the harbor of Alexandria in Egypt (Halim 1960). Species belong to the genus *Alexandrium* are considered as cosmopolitan dinoflagellates because of their wide distribution and bloom-forming capacity in sub-arctic, temperate, tropical and subtropical zones (Taylor et al. 2003). Their life cycle is comprised of a pelagic growth phase and a benthic resting stage by a hypnozygotic cyst formation. This capacity of such resting cyst formation has given the best adaptation scope to *Alexandrium* in different environment and even to disperse worldwide by ballast water (Hallegraeff & Bolch

1992; Kremp et al. 2018). The germination and encystment have significant role in bloom dynamics like seeding and bloom initiation, genetic diversity etc (Kremp et al. 2016; Rengefors & Anderson 1998; Sjöqvist et al. 2015) Species of *Alexandrium* are tend to form seasonal blooms. It has been identified that in Australian coastal waters, the species *A. pacificum* becomes abundant in spring/summer, *A. catenella* in winter/spring, *A. minutum* in early summer/autumn (Ajani, Harwood & Murray 2017). These organisms can also produce bioluminescence (Cusick & Widder 2020). Like the toxins, bioluminescence appears to have a protective effect for some *Alexandrium* species in reducing grazing impacts (Cusick & Widder 2014; Cusick & Widder 2020). Though species of *Alexandrium* are widely known for their harmful activity, their beneficial role has also been discovered. *A. pacificum* and *A. minutum* are reported to produce dimethylsulfopropionate (DMSP) which may influence to balance the climate and the ecosystem restructuring by DMS emission (Caruana et al. 2020).

Species of *Alexandrium* are armored, marine, photosynthetic, planktonic dinoflagellates (Balech 1995). *Alexandrium* cells are covered with thecal plates made up of cellulose. *Alexandrium* species are considered as medium sized marine phytoplankton with a 20- 80 µm length in general (Hoppenrath, Elbrächter & Drebes 2009). Some of the species may produce chains of 4 or more cells (*A. pacificum*) whilst, some species form pairs (*A. tamarense*) (Hoppenrath, Elbrächter & Drebes 2009). *Alexandrium* can reproduce asexually by binary fission (Hoppenrath, Elbrächter & Drebes 2009). *Alexandrium* species also have a sexual life cycle which can be induced by nutrient depletion in laboratory conditions, though the scenario may be different in the natural environment (Probert 1999).

The optimal growth temperature may vary among different *Alexandrium* species. Maximum growth of a strain of *A. fundyense* has been found to be approximately 15˚C and may able to grow within a temperature range from 5 to 20˚C (Natsuike et al. 2017) in one study, whereas the optimal growth temperature for *A. tamiyavanichii* is 25˚C (Lim et al. 2006). The salinity tolerance also varies from species to species (Lim & Ogata 2005). *A. tamarense* can grow across a wide range of salinities from 15 to 30 psu (practical salinity unit) (Lim & Ogata 2005).

To date, about 34 species of *Alexandrium* have been recorded worldwide, of which around 11 species can potentially produce PSTs. The PST producing strains and their toxicity is shown in Table 4.

Table 4: List of Paralytic Shellfish Toxin-producing *Alexandrium* species, toxin produced by them and references showing their toxin production

Species	**Reference**	**Toxins**	**References for toxin production**	**Comments on toxin production**
Alexandrium affine (Inoue et Fukuyo)	(Balech 1985)	Different analogues of saxitoxin	(Nguyen-Ngoc 2004)	Mostly non-toxic (Hallegraeff et al. 1991; Stüken et al. 2011)
A. andersoni	(Balech 1990)	Different analogues of saxitoxin	(Ciminiello et al. 2000)	Mostly non-toxic (Orr et al. 2011; Stüken et al. 2011)
A. australiense Sh. Murray *(A. tamarense* Group V*)*	(John et al. 2014)	Different analogues of saxitoxin	(John et al. 2014)	Both toxic and non-toxic strains are present. Only one strain has been identified as toxic (John et al. 2014)
A. catenella (Whedon et Kofoid) *(A. tamarense* Group I)	(Balech 1985)	Different analogues of saxitoxin	(Lilly, Halanych & Anderson 2007)	Almost all of strains are reported as toxic. One non toxic strain has been reported (John et al. 2014)
A. fragae	(Branco et al. 2020)	Different analogues of saxitoxin	(Branco et al. 2020)	Toxic strains have been reported so far.

A. leei	(Balech 1985)	Different analogues of saxitoxin	(Tang, Kong & Holmes 2007)	It was found as non-toxic in other study (Usup et al. 2002)
A. minutum	(Halim 1960)	Different analogues of saxitoxin	(Chang et al. 1997)	Both toxic and non-toxic strains are present (Touzet, Franco & Raine 2007; Yang et al. 2010)
A. ostenfeldii (Paulsen)	(Balech 1985)	Different analogues of saxitoxin, Spirolides	(Anderson, Cembella & Hallegraeff 2012; MacKenzie et al. 2004)	Both toxic and non-toxic strains are present (Suikkanen et al. 2013)
A. pacificum Litaker *(A. tamarense* Group IV)	Litaker 2014	Different analogues of saxitoxin	Hallegraeff & Lucas, 1988	All strains are toxic (John et al. 2014)
A. tamiyavanichi	Balech 1994	Different analogues of saxitoxin	Hashimoto et al., 2002	All strains are toxic
A. taylori	Balech 1994	Different analogues of saxitoxin	Balech 1994	Usually non-toxic (Anderson et al., 2012)

1.4.1.1 Morphology and phylogenetics of the genus *Alexandrium*

Alexandrium is usually subdivided into species depending on distinguishing morphological features of the thecal plates which may include cell size, differences of shape and ornamentation of the thecal plates, presence or absence of ventral pore, the presence or absence of sulcal plates, chain forming capacity, the apical pore complex’s shape, the size and shape of 1’ and 6’ plates (Balech 1985). The plate pattern for the species follow this formula: APC (apical pore complex), 4', 6", 5''', 2'''', 6C, 9–10S (Balech 1985) (Figure 13).

Beside morphological identification, ITS/5.8S genetic distances, ITS2 compensatory base changes, mating compatibilities, toxicity, presence of *sxt*A gene, and rDNA phylogenies are used for species differentiation. Multiple regions of rDNA such as the large subunit (LSU), internal transcribed spacer region (ITS/5.8S) or the small subunit unit (SSU) are used for phylogenetic identification.

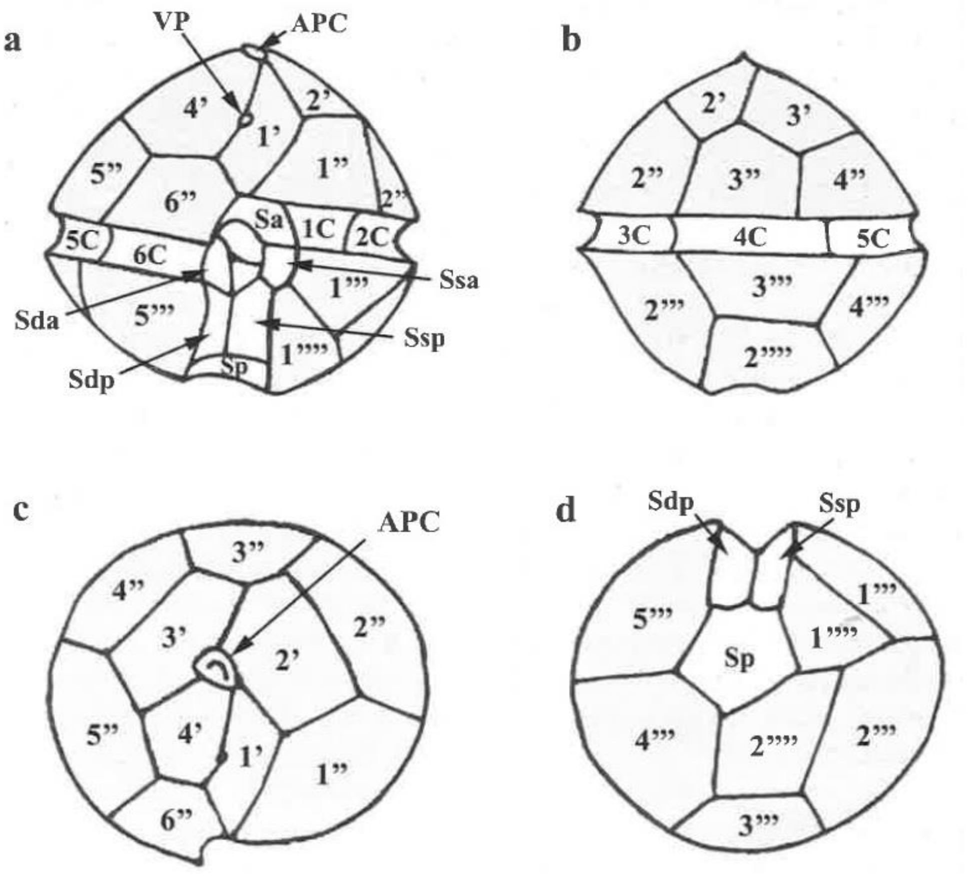


Figure 13: Line diagram of the genus *Alexandrium.* a: Ventral, b: Dorsal, c: Epitheca, d: Hypotheca. APC= Apical pore complex, VP =Ventral pore, C = cingular plates, S = sulcal

plates, 1’-4’ = apical plates, 1’’-6’’ = precingular plates, 1’’’-5’’’ = postcingular plates, 1’’’’-2’’’’= antapical plates (Adapted from (Hoppenrath, Elbrächter & Drebes 2009).

1.4.2 Gymnodinium catenatum

Gymnodinium catenatum has also been reported as a PST producer (Morey-Gaines 1982). It is an athecate species and which can form chains (Hallegraeff, Steffensen & Wetherbee 1988) helping to differentiate it from other *Gymnodinium* species. It is the only species of *Gymnodinium* that produces PSTs (Oshima et al. 1987) and specifically produces some unique analogous of saxitoxin GC1, GC2, GC3 (Negri et al. 2007).

Gymnodinium catenatum was first reported from the Gulf of California in 1943 (Graham 1943), was implicated in a PSP outbreak in the Galician Rias in Spain (Estrada, Sanchez & Fraga 1984), and again from Mexico in 1979 (Oshima et al. 1987). Though other species of *Gymnodinium* were reported before 1973 in Australia (Hallegraeff 1992), *Gymnodinium catenatum* was not reported from Australia until the 1980s (Hallegraeff, McCausland & Brown 1995). *Gymnodinium catenatum* is hypothesized to have been introduced into Australian waters via ships’ ballast water (Hallegraeff & Bolch 1992). It is now widely distributed around the eastern part of Tasmania and is also present in New South Wales (Bolch & de Salas 2007).

1.4.3 Pyrodinium bahamense

Pyrodinium bahamense is a very significant PSP causing species and is responsible for the most bloom-induced fatalities throughout the south-east Asia region (Furio et al. 2012; Usup et al. 2012). About 200 fatal incidents were recorded in southeast Asia caused by the consumption of bivalves containing paralytic shellfish toxins produced by this dinoflagellate (Usup, Kulis & Anderson 1994). It has also emerged as a potential threat to the Pacific and Atlantic coasts of central America, including Florida (Usup et al. 2012). *Pyrodinium bahamense* was first identified in Papua New Guinea in 1972 (Maclean 1989). Although there is no evidence of the existence of this motile plankton in Australia waters to date (Hallegraeff 1992), a cyst fossil study from the port of Darwin suggests that it was present in northern Australia around 120,000 years ago (Hallegraeff 1992).

Pyrodinium bahamense cells are sub-spherical to ellipsoidal, and highly ornamented with an apical projection and node and anterior projections (Figure 12). Blooms of *Pyrodinium* usually form at temperatures 25˚C to 28˚C and high salinities 33‰ to 35‰ (Azanza and Taylor, 2001). Tropical oceans in particular, mangrove-fringed coastal waters of the Atlantic and Indo-West Pacific, is their current niche (Hallegraeff, 2010). There are two varieties of *Pyrodinium bahamense*-- variety that produces PSTs, *P.* var. *compressum* and the other variety, *P.* var. *bahamense*, which does not produce PSTs (Usup et al. 2012).

1.5 Dinoflagellate genetics

Dinoflagellates are considered to be a highly diverse group of microalgae with ecological and economic significance. They are also considered to be a genetically unique group of eukaryotes, as they possess unusual nuclear and organelle genomic organisations and genetic regulation patterns (Figure 14). Their incredibly complex and unique genomic features have inhibited our ability to investigate dinoflagellate genetics easily. Dinoflagellates have amongst the largest known genomes of any group of species, from 1.5 Gbp in *Symbiodinium* to 185 Gbp in *Lingulodinium polyedrum,* and compriseing approximately 3 to 250 pg DNA cell^{-1} (LaJeunesse et al. 2005; Spector 1984; Wang et al. 2014). It has also been observed that the genome size exhibits a positive correlation with cell size in dinoflagellates (Lin 2011), howver this large size is not the result of extreme polyploidy or the proliferation of repetitive DNA (Allen et al. 1975; Davies, Jakobsen & Nordby 1988). In *Heterocapsa triquetra,* it has been identified that about 89.5% of the sequences are non-repetitive, as well as non-coding DNA (McEwan et al. 2008). Furthermore only 0.05%-1.8% of the total genomic DNA are protein coding genes (Lin 2011).

Most dinoflagellate nuclei possess some distinctive characters, such as the presence of dinokaryotic nuclei during some, or all stages, of the life cycle,; permanently condensed identical chromosomes which may vary in number from 24 to 220 within species; nuclear envelop attached chromosomes; and a lack of nucleosomes (Rizzo 2003; Rizzo & Noodén 1972). Dinoflagellate genomes are combined with histone like protein (HLPs) along with a high DNA content (DNA: Protein = 10:1) (Davies, Jakobsen & Nordby 1988; Wong et al. 2003). A relatively high guanine and cytosine (G+C) has been reported to be found in the nuclear DNA with a variation of 45% to 70% (Irwin et al. 2018; Stüken et al. 2011). Another distinguishing feature is the substitution of around 12 - 70% of thymine or cytosine by the methylated nucleobase 5'-hydroxymethyluracil (5-meU) and 5-methyl cytosine respectively (Lin 2011).

Many genes are found to be present as multiple copies in tandem arrays varying from 20-30 to 10^5 (Bachvaroff & Place 2008; Galluzzi et al. 2011). Some dinoflagellates have these tandem repeats with a very low intron density or even lack of introns which are observed as a highly expressed class gene (Bachvaroff & Place 2008; Jaeckisch et al. 2011). On the other hand some other genes have been reported with a high intron density which are considered as less expressed genes (Bachvaroff & Place 2008). Several intron-less genes in dinoflagellates may have resulted from a *trans*-regulatory process, known as retroposition (Beauchemin et al. 2012). The introduction of a conserved 22 bp spliced leader (SL) sequence at the 5' end of transcribed mRNA may also have resulted from this *trans*-regulatory process (Lidie & Van Dolah 2007; Zhang, Bhattacharya & Lin 2007). *Trans*-splicing of SL and polyadenylation help to convert the polycistronic pre-mRNA into a monocistronic form and regulate gene expression (Verma et al. 2019; Zhang, Bhattacharya & Lin 2007). These *trans* spliced monocistronic mRNA make the pool available for translation (Morey et al. 2011). Promoters are reported to be absent in most of the individual genes which results in the post-transcriptional regulation of gene expression (Ouellette & Papadopoulou 2009). Only a few (approximately 5-30%) genes are found to be regulated at the transcription level (Johnson et al. 2012). A recent discovery has been the presence of the protein from eIF4E (eukaryotic translation initiation factor 4E) family in dinoflagellates which provides the possibility of the translational control of gene expression in dinoflagellates (Jones et al. 2015). Another unique finding for the dinoflagellate genome is the common *cis*-regulatory element, TATA boxes, have been replaced by the presence of TTTT(G) (Lin et al. 2015).

Beside nuclear genomes, organelle genomes (both plastid and mitochondrial) are also identified as different in dinoflagellates compared to other eukaryotes. The plastid genes appear as plasmid-like 'minicircles' which can encode only 3 to 4 proteins (Howe, Nisbet & Barbrook

2008; Zhang, Green & Cavalier-Smith 1999). Organelle genomes may also present in several copies and most chloroplast and mitochondrial genes have been transferred to the nucleus ((Howe, Nisbet & Barbrook 2008; Murray et al. 2016; Zhang, Green & Cavalier-Smith 1999).

In summary, dinoflagellates possess a complex genetic machinery with large genome and unusual regulation systems which made it difficult to get the genomic level understanding of different important biosynthetic pathways.

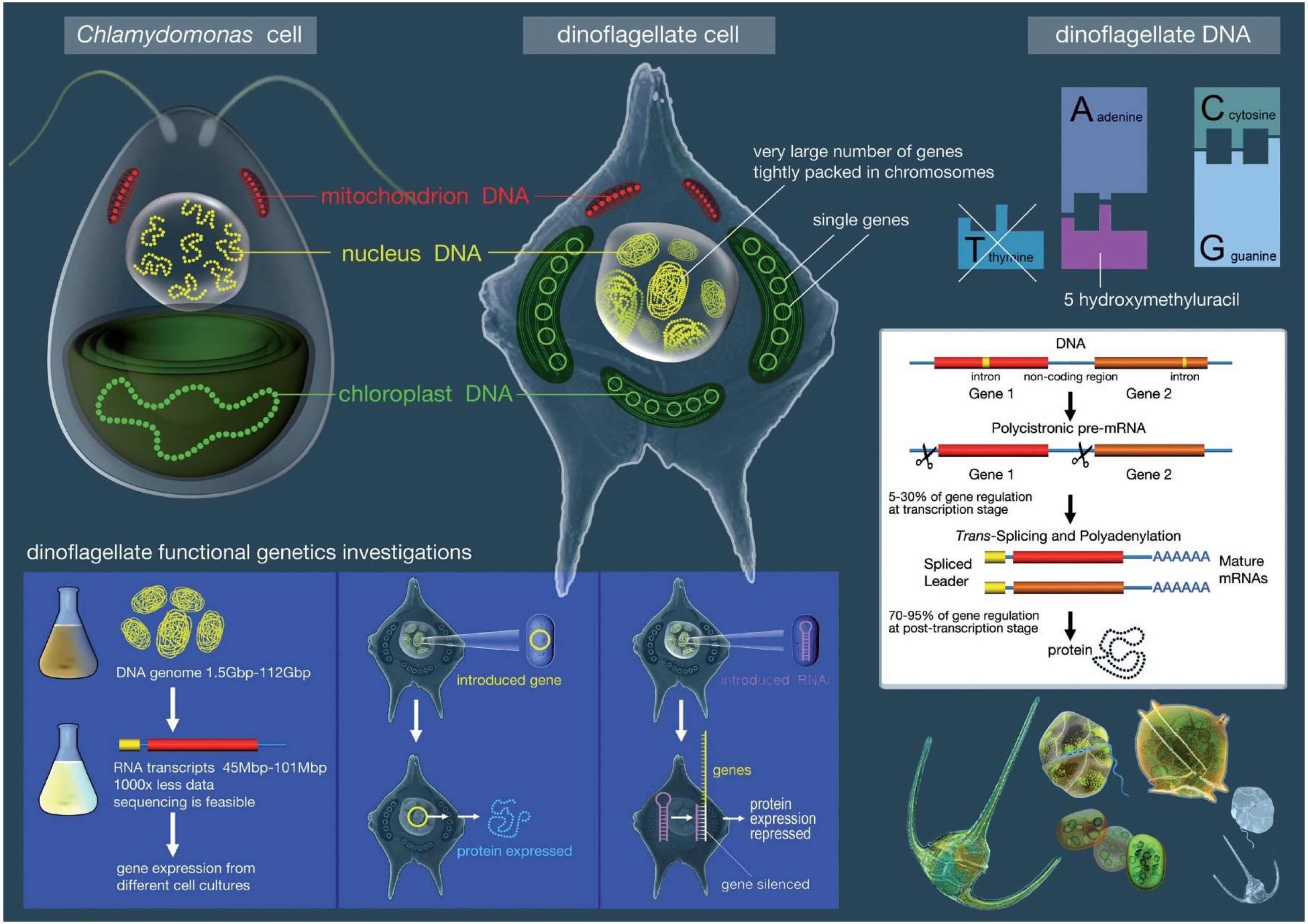

Chlamydomonas cell
dinoflagellate cell
dinoflagellate DNA
mitochondrion DNA
nucleus DNA
chloroplast DNA
very large number of genes tightly packed in chromosomes
single genes
A adenine
C cytosine
T thymine
G guanine
5 hydroxymethyluracil
DNA
intron
non-coding region
intron
Gene 1
Gene 2
Polycistronic pre-mRNA
Gene 1
Gene 2
5-30% of gene regulation at transcription stage
Trans-Splicing and Polyadenylation
Spliced Leader
AAAAAA
AAAAAA
Mature mRNAs
70-95% of gene regulation at post-transcription stage
protein
dinoflagellate functional genetics investigations
DNA genome 1.5Gbp-112Gbp
RNA transcripts 45Mbp-101Mbp
1000x less data
sequencing is feasible
gene expression from different cell cultures
introduced gene
protein expressed
introduced RNAi
genes
protein expression repressed
gene silenced

Figure 14: The genetic differences between a typical dinoflagellate and other phytoplankton such as the cholorophyte *Chlamydomonas reinhardtii* (Adapted from (Murray et al. 2016)

1.6 PST biosynthesis pathway in dinoflagellates and associated gene cluster

The PST biosynthetic process was investigated by radioisotope labelled precursor incorporation in the 1980s for the first time (Shimizu et al. 1984). The putatively involved *sxt* gene cluster was first described from cyanobacterial genera, though the PSTs appeared to be synthesized through a similar biosynthetic pathway using the same precursors acetic acid, arginine, and S-adenosyl methionine (SAM) in both prokaryotic cyanobacteria and eukaryotic dinoflagellates (Kellmann et al. 2008; Tsuchiya et al. 2017).

Fourteen *sxt* genes (*sxt*A – *sxt*I, *sxt*P – *sxt*S and *sxt*U) have been identified in PST producing cyanobacteria and are considered as “core genes” (Kellmann et al. 2008; Murray et al. 2011). Among them, the direct involvement of eight of the core *sxt* genes have been reported (Kellmann et al. 2008). Besides the core genes, there are some other gene groups have been identified, namely, regulatory genes, tailoring genes, and transporter genes (Akbar et al. 2018). In cyanobacteria, it has been observed that the recognised *sxt* gene cluster for this toxin biosynthesis is encoded by 31 Open Reading Frames (ORFs) assigned to 26 proteins (Mihali, Kellmann & Neilan 2009; Moustafa et al. 2009).

The identification of the *sxt* genes in dinoflagellates became easier after the discovery of these genes in the cyanobacterial genera (Hackett et al. 2013; Murray et al. 2015; Orr et al. 2013b; Stüken et al. 2011; Zhang et al. 2014). With the help of cyanobacterial *sxt* gene sequences, and use of transcriptomic annotation, PCR, and amplicon sequencing technologies, the identification and characterization of *sxt* homologs in dinoflagellate species was possible (Stüken et al. 2011, Murray et al. 2015, Orr et al. 2013, Hackett et al. 2012, Zhang et al. 2014). These *sxt* genes have the characteristics similar to other dinoflagellate genes; i.e., a high (~65%) GC content, expressed as monocistronic transcripts with a 5’ spliced leader sequence and a 3’eukaryotic poly-A tail (Orr et al. 2013).

In the PST biosynthesis pathway, the enzyme coded by the gene *sxtA* has been identified as an initiator enzyme in both cyanobacteria and dinoflagellate (Kellmann et al. 2008; Tsuchiya et al. 2014). It contains four catalytic domains, encoded by *sxtA1* (SAM-dependent methyltransferase, MT), *sxtA2* (GCN 5-related N-acetyltransferase, ACT), *sxtA3* (acyl carrier protein, ACP), and *sxtA4* (8-amino-7-oxononanoate synthase like class II aminotransferase, AONS) (Kellmann et al. 2008). Binding of acetyl-CoA derived acetyl with the ACP acetyl-ACP, which converts into propionyl-ACP after methylation of the acetate (Kellmann et al. 2008). Claisen condensation between arginine and propionyl-ACP happens to produce 4-amino-3-oxo-guanidinoheptane, which is denotated as 'Compound A' in figure 15 (Kellmann et al. 2008).

Depending on the presence or absence of domain *sxtA4*, two isoforms of *sxtA* are found in dinoflagellates. One is the long transcript containing *sxtA1- A4* which is active in catalyzing STX biosynthesis (Murray et al. 2011a) and other is the short one where *sxtA4* domain is absent (Stüken et al. 2011).

sxtG is the second core PST biosynthesis gene which encodes amidinotransferase (Orr et al. 2013) (Figure 15). Dinoflagellate *sxtG* is homologous to its cyanobacterial one with acquired eukaryotic features over the course of its evolution. The enzyme catalyses the second reaction step by transferring a guanidine group from a second arginine to the growing SXT backbone forming Compound B (Stüken et al. 2011). It has been reported that *sxtG* transcripts are monocistronic and contain a poly A tail at the 3' end and spliced leader sequence at the 5′ ends of the mRNA (Orr et al. 2013). Similar to *sxt*A, the GC content is higher in dinoflagellate *sxt*G than in the cyanobacterial one (Stüken et al. 2011). Though introns are generally not common in dinoflagellate genes, introns of variable lengths have been reported in *sxtG* in some *Alexandrium* spp. (Orr et al. 2013).

Both *sxtA* and *sxtG* have been studied at the genomic and transcriptomic level in dinoflagellates (REFS). Apart from these, the other toxin-related genes have not been characterized at the genomic level, and are known from transcriptomic studies involving several PST-producing dinoflagellate species (Stüken et al. 2011; Murray et al. 2015; Orr et al. 2013; Hackett et al.

2012; Zhang et al. 2014, 2017) (Table 05) For transcriptomic studies of saxitoxin producing dinoflagellate, MPSS, Roche 454 GS FLX, Microarray, Illumina HiSeq were commonly used as platform (Bi et al. 2019).

Table 05. Putative sxt genes in the saxitoxin producing dinoflagellates species.

Gene	Function *	Dinoflagellate Species	Reported from Transcriptome	Reported from Genome
Core sxt Genes				
sxtA (sxtA4)	Aspartate aminotransferase	*Alexandrium australiense*	na	Yes (Stüken et al 2011, Murray et al 2015)
		Alexandrium catenella	Yes (Stüken et al 2011, Hackett et al 2012)	Yes (Stüken et al 2011, John et al 2014, Murray et al 2015]
		Alexandrium minutum	Yes (Stüken et al 2011)	Yes (Stüken et al 2011, Murray et al 2015)
		Alexandrium ostenfeldii	na	Yes (Suikknen et al 2013)
		Alexandrium pacificum	Yes (Murray et al 2015, , Hackett et al 2012, Zhang et al 2014, Zhang et al 2017)	Yes (Stüken et al 2011, Murray et al 2015)
		Alexandrium tamarense	No (John et al 2014, Hackett et al 2012]	Yes (Stüken et al 2011; No (John et al 2014, Murray et al 2015)
		Gymnodinium catenatum	Yes (Murray et al 2015)	Yes (Stüken et al 2011, Mendoza-Flores et al 2018)
		Pyrodinium bahamense	Yes (Murray et al 2015)	na
sxtG	Amidinotransferase	*Alexandrium affine*	No (Orr et al 2013)	Yes (Orr et al 2013)
		Alexandrium andersoni	No (Orr et al 2013)	Yes (Orr et al 2013)
		Alexandrium australiense	Yes (Orr et al 2013)	Yes (Orr et al 2013)
		Alexandrium catenella	Yes (Stüken et al 2011)	Yes (Orr et al 2013)
		Alexandrium insuetum	No (Orr et al 2013)	Yes (Orr et al 2013)
		Alexandrium minutum	Yes (Stüken et al 2011)	Yes (Orr et al 2013)

		Alexandrium pacificum	Yes (Orr et al 2013, Hackett et al 2012, Zhang et al 2014, Zhang et al 2017)	Yes (Orr et al 2013)
		Gymnodinium catenatum	Yes (Murray et al 2015, Hackett et al 2012)	Yes (Orr et al 2013)
		Pyrodinium bahamense	Yes (Murray et al 2015, Hackett et al 2012)	na
sxtB	Cytidine deaminase	*Alexandrium catenella*	Yes (Stüken et al 2011)	na
		Alexandrium minutum	Yes (Stüken et al 2011)	na
		Alexandrium pacificum	Yes (Hackett et al 2012, Zhang et al 2014, Zhang et al 2017)	na
sxtD	Sterole desaturase	*Alexandrium pacificum*	Yes (Hackett et al 2012 , Zhang et al 2014, Zhang et al 2017)	na
sxtS	Phytanoyl-CoA dioxygenase	*Alexandrium catenella*	No (Stüken et al 2011); Yes (Hackett et al 2012)	na
		Alexandrium minutum	Yes (Stüken et al 2011)	na
		Alexandrium pacificum	Yes (Hackett et al 2012)	na
		Gymnodinium catenatum	Yes (Hackett et al 2012)	na
		Pyrodinium bahamense	Yes (Hackett et al 2012)	na
sxtU	Alcohol dehydrogenase	*Alexandrium catenella*	Yes (Stüken et al 2011, Hackett et al 2012)	na
		Alexandrium minutum	Yes (Stüken et al 2011)	na
		Alexandrium pacificum	Yes (Hackett et al 2012 , Zhang et al 2014, Zhang et al 2017)	na
		Pyrodinium bahamense	Yes (Hackett et al 2012)	na
sxtH/T	Phenylpropionate dioxygenase	*Alexandrium catenella*	Yes (Stüken et al 2011, Hackett et al 2012)	na
		Alexandrium minutum	Yes (Stüken et al 2011)	na
		Alexandrium pacificum	Yes (Hackett et al 2012, Zhang et al 2014); Yes as H/T/DIOX (Zhang et al 2017)	na
sxtI	O-carbamoyl transferase	*Alexandrium catenella*	Yes (Stüken et al 2011)	na
		Alexandrium minutum	Yes (Stüken et al 2011)	na
		Alexandrium pacificum	Yes (Hackett et al 2012, Zhang et al 2014, Zhang et al 2017)	na
		Pyrodinium bahamense	Yes (Hackett et al 2012)	na
sxtF/M	Multidrug efflux protein	*Alexandrium catenella*	Yes (Stüken et al 2011)	na
		Alexandrium minutum	Yes (Stüken et al 2011)	na
		Alexandrium pacificum	Yes (Hackett et al 2012, Zhang et al 2014, Zhang et al 2017)	na
sxtP	STX binding protein	*Alexandrium catenella*	Yes (Hackett et al 2012)	na
		Alexandrium pacificum	Yes (Hackett et al 2012, Zhang et al 2017)	na

Other sxt Genes				
sxtL	GDSL lipase	*Alexandrium pacificum*	Yes (Hackett et al 2012)	na
sxtN	Sulfotransferase	*Alexandrium pacificum*	Yes (Hackett et al 2012)	na
sxtO	Adenylylsulfate kinase	*Alexandrium pacificum*	Yes (Zhang et al 2014, Zhang et al 2017)	na
sxtR	Acetyl CoA N-acyltransferase	*Alexandrium catenella*	Yes (Stüken et al 2011)	na
		Alexandrium minutum	Yes (Stüken et al 2011)	na
sxtW	Ferredoxin	*Alexandrium pacificum*	Yes (Zhang et al 2017)	na
sxtX	Cephalosporine hydroxylase	*Alexandrium pacificum*	Yes (Hackett et al 2012, Zhang et al 2014, 2017)	na
		Pyrodinium bahamense	Yes (Hackett et al 2012)	na
sxtZ	Histidine kinase	*Alexandrium pacificum*	Yes (Zhang et al 2014, 2017)	na
sxtPER	Permease	*Alexandrium pacificum*	Yes (Zhang et al 2017)	na
sxtACT	Acetylase	*Alexandrium pacificum*	Yes JoZhang et al 2017)	na

The third step of the PST biosynthesis pathway is a heterocyclization reaction which is catalysed by the *sxtB* encoded enzyme, cytidine deaminase, and forms the first heterocyclic compound (Kellmann et al 2008). Between two most distal carbons, a double bond is formed by the catalytic action of the sterole-desaturase related enzyme, gene product of *sxtD*. The subsequent synthesis of the second and third heterocycles via epoxidation and aldehyde formation happen by *sxtS* encoded product (Mihali et al 2009, Kellmann et al, 2008). Following these steps, the enzymes *sxtU* and *sxtH/T* work in sequence to produce the first complete PST analogue, dcSTX. Carbomylation by sxtI produces the parent compound STX (Mihali et al 2009, Kellmann et al, 2008). The biosynthesis pathway has been shown in Figure 15.

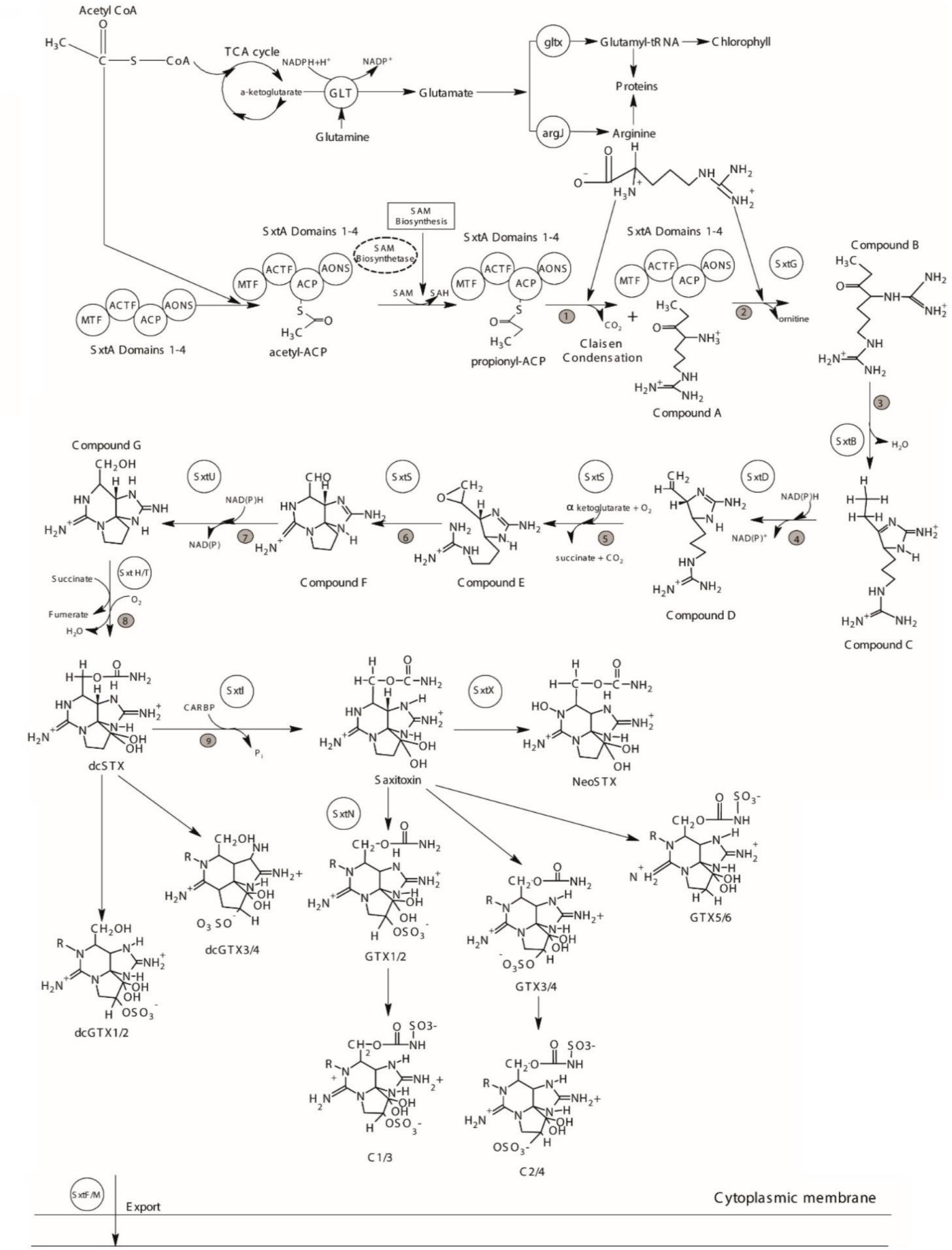

Acetyl CoA
CoA
TCA cycle
NADPH+H+
NADP+
a-ketoglutarate
GLT
Glutamine
Glutamate
gltx
Glutamyl-tRNA
Chlorophyll
Proteins
argJ
Arginine
SAM Biosynthesis
SAM Biosynthetase
SAM
SAH
SxtA Domains 1-4
MTF
ACTF
ACP
AONS
acetyl-ACP
propionyl-ACP
1
CO2
Claisen Condensation
Compound A
SxtG
2
ornitine
Compound B
3
SxtB
H2O
Compound C
4
NAD(P)H
NAD(P)+
SxtD
Compound D
5
SxtS
α ketoglutarate + O2
succinate + CO2
Compound E
6
SxtS
Compound F
7
SxtU
NAD(P)H
NAD(P)
Compound G
Sxt H/T
Succinate
O2
Fumerate
H2O
8
dcSTX
SxtI
CARBP
9
Pi
Saxitoxin
SxtX
NeoSTX
SxtN
dcGTX1/2
dcGTX3/4
GTX1/2
GTX3/4
GTX5/6
C1/3
C2/4
SxtF/M
Export
Cytoplasmic membrane

Figure 15: Revised biosynthetic pathway of PSTs in dinoflagellates (modified from Shimizy 1993, Mihali et al 2009, Kellmann et al 2008, Zhang et al 2014, 2017, Neilan 2014). 1: Claisen comdensation; 2: amidino transfer, 3: heterocyclization; 4: desaturation (Double bond formation) 5: epoxidation of the new double bond; 6: aldehyde formation; 7: terminal aldehyde reduction; 8: dihydroxylation and 9: carbamoylation (Adapted from Verma et al 2019)

1.7 Concepts on *sxt* Gene Regulation and *sxt* gene evolution

The possibility of transcriptional control of PST biosynthesis has been investigated in *Alexandrium* spp., and the hypothesis has been supported by the discovery of putative sxtZ homologs, a gene which in cyanobacteria is thought to be involved in the transcriptional regulation of STX biosynthesis (Zhang et al 2014). The presence of a spliced leader sequence in *sxtA4* and *sxtG* genes gives the additional support on the post-transcriptional regulation of saxitoxin biosynthesis (Stüken et al 2011, Orr et al, 2013,Danks et al 2015, Biltar et al 2013).

Much genomic and transcriptomic level research has been performed to identify the obligations of particular genes in the PST biosynthesis pathway. Until now, it is believed that the domain *sxtA4* is the main key in the PST biosynthesis pathway. The molecular detection primers for toxic dinoflagellates are usually designed, based on the sequence of domain A4 as domain A4 is thought to be crucial for STX biosynthesis (Murray et al., 2011b). It has been reported that dinoflagellate genera or families which do not produce STX appear to lack the domain *sxtA4* (Stüken et al.,2011; Orr et al., 2013; Murray et al., 2014; John et al., 2014). Later on the presence of this domain was identified in the genome of non-toxic mutant of *A. pacificum*, though the expression was not observed in the mRNA during differential gene expression analysis (Zhang et al 2014). The absence of the domain sxtA4 was also noted for *A. tamarense* III, whereas this domain is present in the genome (Murray et al 2015). The long *sxtA* transcript is found to express strongly in STX-producing strains of the same species (Murray et al, 2015). Neverthless, there was no significant differential expression of the short *sxtA* transcript detected (excluding sxtA4, as represented in Figure 15) between toxic and non-toxic strains (Zhang et al 2014).

It was assumed that this gene was transferred laterally as the sequences of *sxtA1* to *sxtA3* is similar with deltaproteobacterium sequences and the sequences of *sxtA4* show similarity with actinobacteria (Moustafa et al., 2009). In another study, it was proposed that the origin of the *sxt* gene in dinoflagellates is a single horizontal gene transfer (HGT) event (Murray et al 2015). While Orr et al 2013 proposed the HGT from *Alexandrium* to *Gymnodinium catenatum*, another breakthrough discovery has challenged this proposal. Along with the earlier evolution report of *G. catenatum* (around of 150-140 MYA) than *Alexandrium* (late Cretaceous 77MYA), the recent discovery on the presence of *sxtA1* paralogs in two other Gymnodiniales species (*Karenia brevis* and *Pelagodinium beii*), have confirmed that the HGT event might have happened before the divergence of Gymnodiniales and Gonyaulacales (Mendoza- Flores et al 2018).

In dinoflagellates, instead of a single homolog, a phylogenetic analysis of *sxtA1* revealed that it consisted of three paralogs - clade 1, clade 2 and clade 3, though only one of these copies is linked to PST synthesis (Murray et al. 2015). This incident indicates the presence of gene duplication in dinoflagellates (Murray et al. 2015). The duplication process may vary within the STX producing dinoflagellates, and may lead to the presence of different gene copies within species (Mendoza- Flores et al 2018). Clade 3 is composed of almost every dinoflagellate family and order, while clade 1 has the *Alexandrium* species that are a non-STX synthesizer or have toxic and nontoxic strains (Murray et al., 2015). Clade 2 of *sxtA1* domain is mostly made up of STX producing dinoflagellates (*Alexandrium minutum*, *A. pacificum, A. australianse, A. fundeyense, Pyrodinium bahamense and Gymnodinium catenatum*) with two non-STX producing *Alexandrium* species (*A. margalefi* and *A. pseudogonyaulax*) (Murray et al., 2015). On the otherhand, the *sxtA4* domain was found as homologues and highly conserved in several strains of eight species of STX-producing dinoflagellates (Murray et al., 2015). However, a recent study has identified the presence of two separated clades (a *Gymnodinium catenatum* clade, and an *Alexandrium/ Pyrodinium* clade) in a *sxtA4* domain based phylogeny (Mendoza-Flores et al 2018). More recently, the presence of *sxtA, sxtG* and *sxtU* have been identified in the non-PST producing *Alexandrium tamutum* with or without exposure to nutrient limitation (phosphate) (Vingiani et al., 2020).

Until recently, *sxtG* was supposed to be absent or it may be transcribed at extremely low levels in the non-PST producing dinoflagellates genera (Orr et al. 2013). Only one highly conserved clade has been identified during the *sxtG* phylogeny which includes all of the PST producing dinoflagellates (Murray et al 2015). *sxtG* transcript has been identified in the non PST producing *Alexandrium tamutum* (Vingiani et al., 2020). It is well supported that both *sxt*A and *sxt*G have been transferred from prokaryotes though the transfer pattern is not clear (Murray et al., 2015).

In a comparative transcriptomic study between non saxitoxin producer *Cochlodinium polykrikoides* and *Alexandrium pacificum* the presence of eight most important STX synthesis gene (*sxtA, sxtB, sxtD, sxtG, sxtH/T, sxtI, sxtS* and *sxtU*) were revealed in *C. polykrikoides,* whereas the core gene *sxtG* was not detected,possibly explaining the absence of saxitoxin production in *C. polykrikoides* (Wang et al 2020a). During the transcriptome analysis of non-STX producing *Scrippsiella trochoidea,* several *sxt* homologues including the short isoform of *sxt*A1-A3 were characterized, which is not related to saxitoxin production (Cooper et al 2016). In another study, the PST biosynthetic core genes *sxtG, sxtH/T, sxtI, sxtS, sxtU, sxtL, sxtN, sxtF/M, sxtP, sxtO, sxtC*, and *sxtV*, were identified in *Prorocentrum micans* and *Amphidinium carterae* which are known as non PST producing organisms (Wang et al 2020b). In the same study, core genes long isoform of *sxtA* and *sxtB* were discovered in *P. micans* and *sxtD, sxtR, sxtX,* and *sxtZ* were found in *A. carterae* separately (Wang et al 2020b). Sequence homology of *sxtA* identified dissimilarities between the of non PST producing dinoflagellate *P. micans* with PST producing *Alexandrium catenella* and *A. pacificum* (Wang et al 2020b).

Along with the presence or absence of *sxt* genes, a study on *A minutum* has suggested positive correlation between the genomic copy numbers of *sxtA4* with a total cellular PST content (Stüken et al., 2015). As both bioluminescence and toxicity are related with grazing deterrents, there might have a correlation between the molecular mechanisms of these two separate function; which has not been described as yet (Cusick & Widder 2020). Together these findings suggest that the PST biosynthesis genes in dinoflagellates are under a complex regulatory system or may be the gene silencing of the saxitoxin biosynthesis gene cluster may interfere in the expression of the non-saxitoxin producing dinoflagellate (Akbar et al 2020).

Several proteomics studies have been performed which provide insights into the mechanisms and possible translational regulation of STX biosynthesis. Correlation between the PST biosynthesis pathway and other carbon and energy utilizing pathways has been observed (Wang et al 2012, Zhang et al 2018). Proteins involved in the translational machinery, photosynthetic pigment production, and toxin biosynthesis with linkages to arginine, which is an important PST precursor molecule, and glutamate biosynthesis, were found to be upregulated during the same stage of the cell cycle (Zhang et al 2018, Shimizu 1986, Taoncher-Oldenburg et al 2000). Proteins involved in bioluminescence have been observed to be upregulated during toxin production (Zhang et al 2018) and found to be downregulated in a non-toxic strain with toxin related proteins (Wang et al 2012). Hence, it can be concluded that the toxin biosynthesis might not be regulated as a single pathway, rather is regulated in concert with other biosynthetic pathways.

1.8 Population genetics and diversity

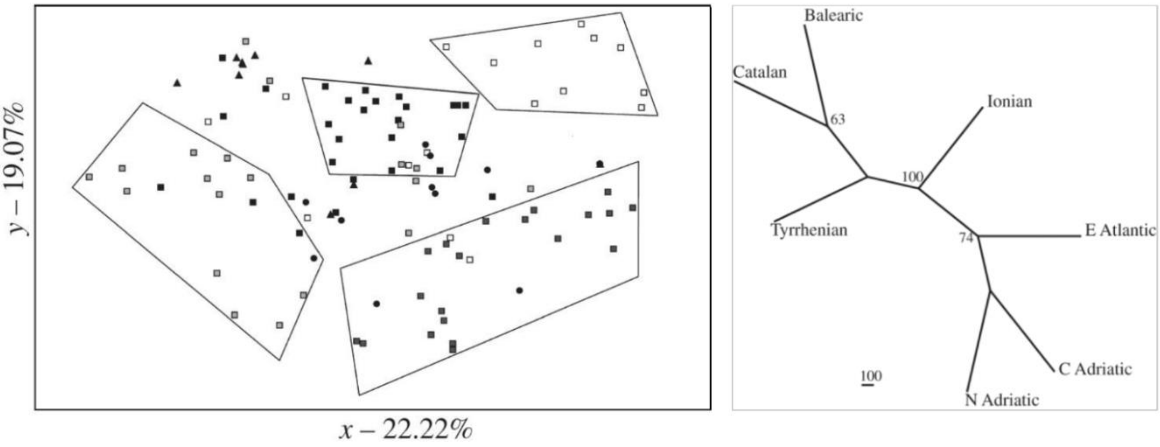
When a group of phytoplankton from a single species occupy the same habitat to live and interbreed, they are considered a population of that specific phytoplankton (Freeland et al., 2011). Different populations may differ from one another genetically, physiologically, and in toxicology (Briand et al., 2004, Brand, 1989, Freeland et al., 2011, Casabianca et al., 2012). Genetic variation within a species could be influenced by certain factors, where genetic drift is the most influential one (Boreo et al., 1996, Freeland et al., 2011). The random alteration of allele frequencies leads to changes in the genetic diversity of the population and species. Non-adaptive evolutionary change may occur when gene flow is absent. Gene flow helps to keep genetic interconnection within a population unit during species evolution; hence, it prevents genetic diversity in that unit (Bohonak, 1999, Freeland et al., 2011).

Marine planktonic populations have been thought to be highly connected, as they have been thought to be easily distributed and transported throughout the world's oceans (Norris, 2000; Palumbi, 2003). They have been thought to have a high dispersal capacity due to passive transport and oceanographic currents and other features (Cermeno and Falkowski, 2009White et al., 2010). In some cases, the presence of physical barriers may prevent genetic diversity (Freeland et al., 2011).

A wide range of techniques are available for understanding the biodiversity of phytoplankton. Ribosomal RNA (‘barcode’) marker regions are used to determine the species-level diversity of a community, but they are not appropriate markers to identify intraspecific variability (Masseret et al., 2009). The methods required to identify population differences are finer scaled, and therefore different approaches are required. Microsatellites (microsats), amplified fragment length polymorphisms (AFLPs), single nucleotide polymorphisms (SNPs), and insertion-deletion polymorphisms (indels) are the common markers that are used in marker-based assays (Mariette et al., 2000, Freeland et al., 2011, Davey and Blaxter, 2011). Microsatellite markers are highly polymorphic genetic markers which are appropriate for analyzing intraspecific diversity (Masseret et al., 2009). RADSeq (Restriction site associated DNA sequencing) is becoming more popular and is considered as the most versatile tool for next-generation sequencing-based population genetic studies (Baird et al., 2008; Hohenlohe et al., 2010; Emerson et al., 2010; Langmead et al., 2009). This method possesses some unique features, as it does not require existing sequence data, and can be used to identify and score thousands of randomly distributed genetic markers. (Davey and Blaxter, 2011).

Microplanktonic populations, in particular, species of *Alexandrium,* are now found in coastal areas all over the world, and rapid dispersal activity may be one important reason behind this current scenario (Hallegraeff, 2010). These microbial populations may be subjected to different environmental stress because of these geographical changes that may lead to genetic changes in the vicariant populations (Hallegraeff, 2010, Casabianca et al., 2012).

In recent studies on a species of *Alexandrium,* it has been shown that barriers to gene flow can exist in oceans, and cause genetic isolation, such as was found in a study of *Alexandrium minutum* populations in the Mediterranean Sea (Figure 16) (Casabianca et al., 2012). Four identical genetically homogenous clusters corresponding to four different seas --- Adriatic, Ionian, Tyrrhenian and Catalan, were identified by a PCoA (Principal coordinate analysis) based on seven microsatellite loci of *Alexandrium minutum* (Figure 16 a) (Casabianca et. al., 2012). Though four population groups from four geographical origins with some allochthonous genotypes from other regional seas individual were demonstrated, samples were collected from seven different sites of the Mediterranean Sea where six sampling areas were well differentiated by NJ dendrogram (Figure 16 b) (Casabianca et. al., 2012).



(a) (b)

Figure 16: (a) Principal coordinate analysis (PCoA) based on 116 isolates of *Alexandrium minutum* typed at seven microsatellite loci. Black square, N Adriatic; dark grey square, C Adriatic; white square, Ionian; light grey square, Tyrrhenian; grey square, Catalan; black circle, Balearic; black triangle, E Atlantic. (b) Neighbour Joining unrooted tree of seven *Alexandrium minutum* sampling sites in the Mediterranean Sea and Atlantic based on a chord distance matrix. Bootstrap values (>60%) are placed at each node (Casabianca et al., 2012).

In the Mediterranean Sea, the coastal topography is responsible for closed circulation that inhibits dispersal between two closest places with geographically straight distance and makes direct cell exchange low (Figure 17) (Casabianca et al., 2012). Internal connectivity between Western Adriatic and Western Mediterranean site is very high due to the presence of two straits that maintains stable cyclonic circulation and make this area as a geographically isolated place (Figure 17) (Casabianca et al., 2012).

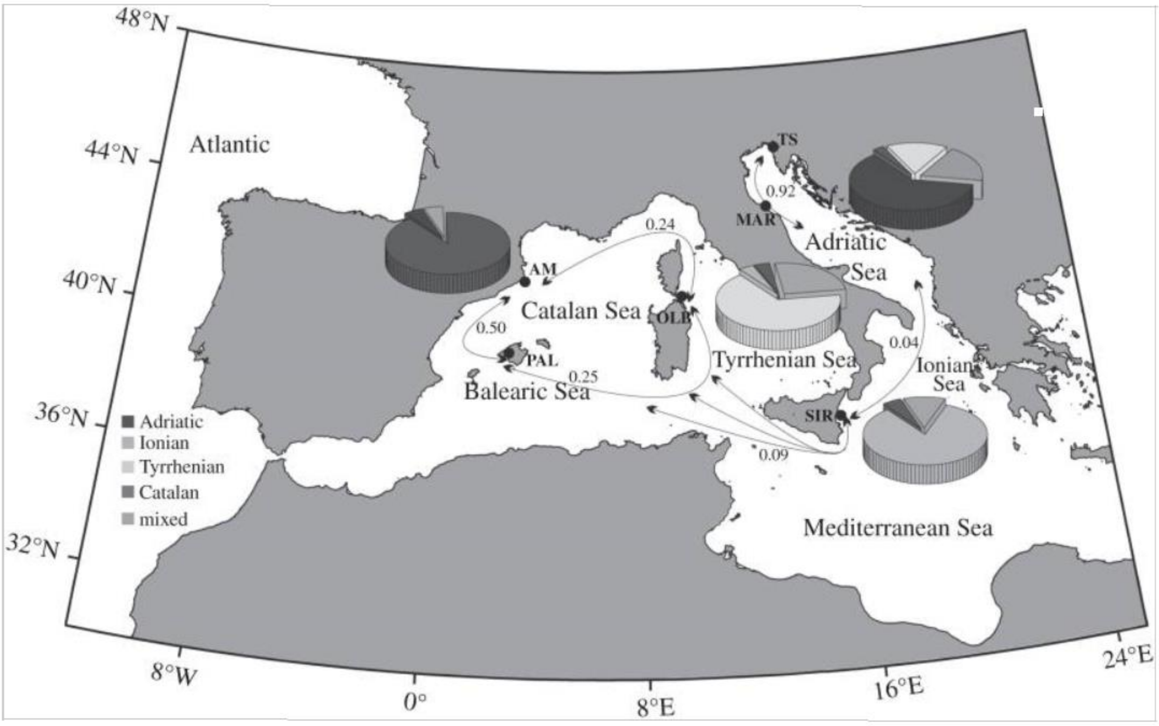


Figure 17: Distribution of different populations of *Alexandrium minutum* and indirect connectivity estimated from different sampling sites in the Mediterranean Sea. The pie chart for each site shows the percentage of individuals, typed at seven microsatellites, assigned to one of the four clusters identified by PCoA and the Bayesian method (Casabianca et al., 2012).

A similar situation, in which barriers to gene flow were found to impact population diversity, was reported in Japanese Pacific coastal areas. The presence of Chita Peninsula geographically separated Mikawa Bay from Hamanako, though the distance is only 20 to 30 km (Figure 18). Hence, Mikawa experience only *Alexandrium tamarense* bloom without any report on the presence of *A. catenella*, though *A. catenella* appeared in Hamanako (Nagai et al., 2006).

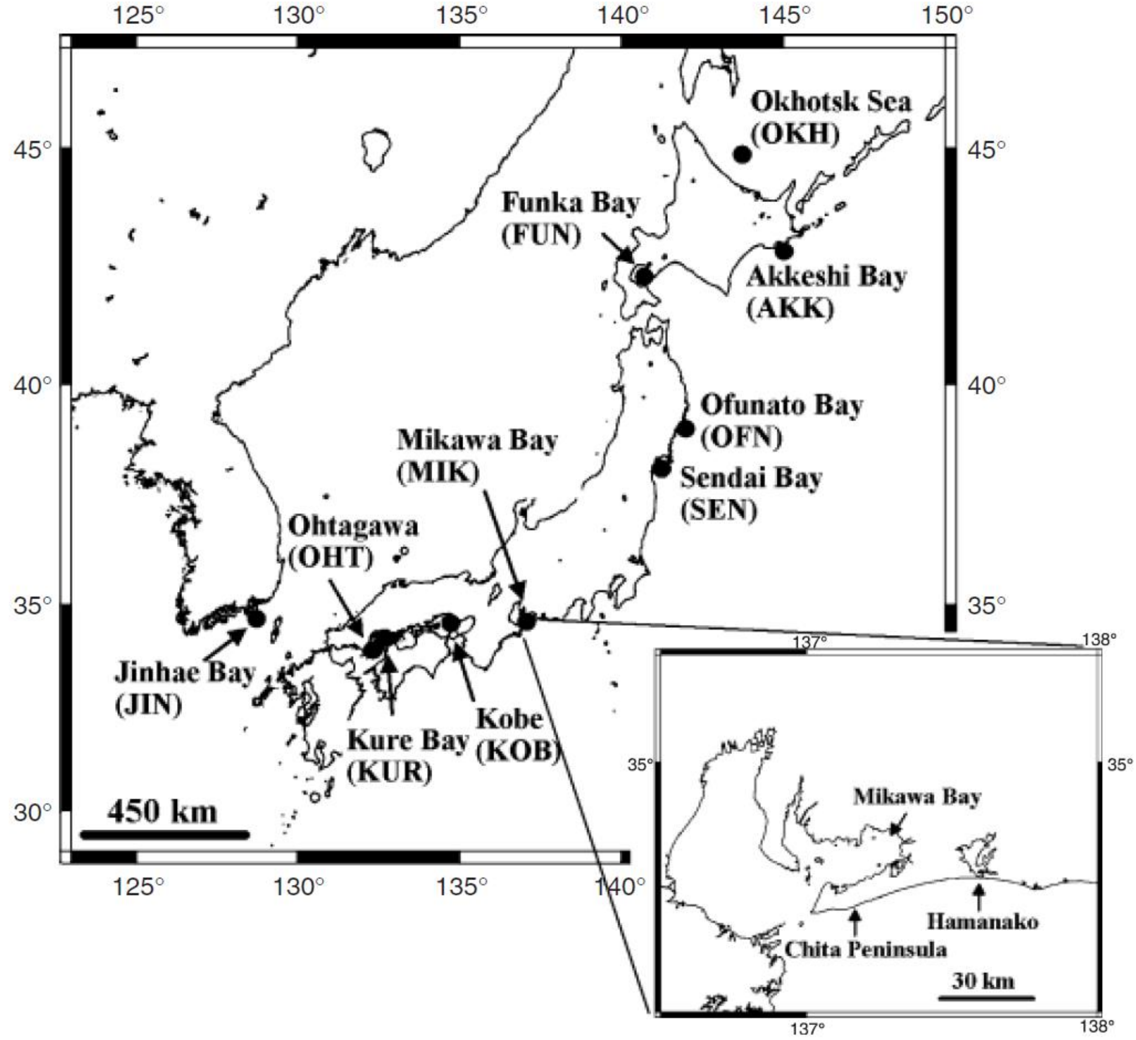


Figure 18: Sampling locations. Locations where seawater and sediment samples containing *Alexandrium tamarense* were obtained. Inset: Geographic location of Mikawa Bay (MIK) and Hamanako in Japan (Nagai et al., 2006).

A population genetic structure and biogeographic study of Japanese *A. tamarense* populations has revealed that genetic distance is correlated with geographic distance, and gene flow between Japanese and Korean coastal areas has been resulted when the genetic structure has been dispersed by human activity like shipping (Nagai et al., 2007). In addition, gene flow is common in Japanese Pacific coastal area. It was thought that the Tsugaru Warm current and the translocation of seed scallop helped the introduction of *A. tamarense* in Ofunato Bay from Funka Bay (Figure 19) as highly similar genotypes were found in a study using mSAT markers, despite significant geographical distances between sites (Nagai et al., 2006). Similar genetic dispersal of *A. tamarense* was reported between Mikawa Bay and Kobe, where human activity such as shipping was assumed as a mode of population transfer and between Kure Bay and Ohtagawa Bay, where daily boat traffic assisted mixing of *A. tamarense* population (Nagai et al., 2006).

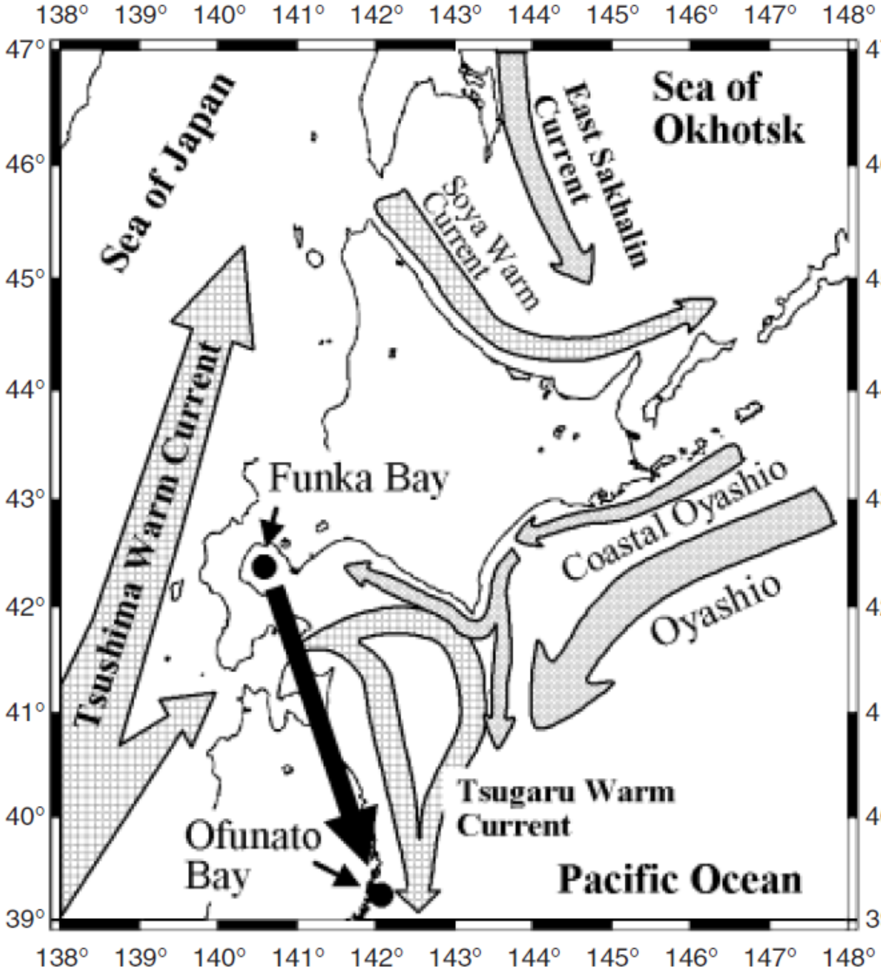


Figure 19: Schematic paths of the warm currents (Tsushima, Soya, and Tsugaru Warm Currents) and cold currents (East Sakhalin Current, Coastal Oyashio, and Oyashio) around Hokkaido (Isoda and kishi 2003; Nagai et al., 2006)

Chapter 2

First Detection of Paralytic Shellfish Toxins from *Alexandrium pacificum* above the Regulatory Limit in Blue Mussels (*Mytilus galloprovincialis*) in New South Wales, Australia

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Authorship Declaration

By signing below, I confirm that for the paper titled "First Detection of Paralytic Shellfish Toxins from *Alexandrium pacificum* above the Regulatory Limit in Blue Mussels (*Mytilus galloprovincialis*) in New South Wales, Australia" and published by the journal "Microorganisms", that

Abanti Barua: Conceptualized, performed the work described in methodology section, formal analysis, data curation, original draft preparation, review and editing.

Penelope Ajani: Conceptualized, performed formal analysis, original draft preparation, review and editing.

Rendy Ruvindy: performed qPCR, performed formal analysis

Hazel Farrell: Conceptualized, Prepared original draft, performed review and editing.

Anthony Zammit: Conceptualized, performed review and editing.

Steve Brett: Performed morphological characterization, data curation, review and editing

David Hill: Performed morphological characterization, data curation, review and editing

Chowdhury Sarowar: Performed toxin analysis and data curation.

Mona Hoppenrath: Performed morphological characterization, review and editing

Shauna A Murray: Conceptualized, prepared original draft, performed review and editing.

Production Note: Signature removed prior to publication.	Production Note: Signature removed prior to publication.	Production Note: Signature removed prior to publication.
Abanti Barua	Penelope Ajani	Rendy Ruvindy
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Hazel Farrell	Anthony Zammit	Steve Brett
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David Hill	Chowdhury Sarowar	Mona Hoppenrath
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6.10.2021

2.1 Abstract

In 2016, 2017 and 2018, elevated levels of the species *Alexandrium pacificum* were detected within a blue mussel (*Mytilus galloprovincialis*) aquaculture area at Twofold Bay on the south coast of New South Wales, Australia. In 2016, the bloom persisted for at least eight weeks and maximum cell concentrations of 89,000 cells L^{-1} of *A. pacificum* were reported. The identity of *A. pacificum* was confirmed using molecular genetic tools (qPCR and amplicon sequencing) and complemented by light and scanning electron microscopy of cultured strains. Maximum reported concentrations of paralytic shellfish toxins (PSTs) in mussel tissue was 7.2 mg/kg PST STX equivalent. Elevated cell concentrations of *A. pacificum* were reported along the adjacent coastal shelf areas, and positive PST results were reported from nearby oyster producing estuaries during 2016. This is the first record of PSTs above the regulatory limit (0.8 mg/kg) in commercial aquaculture in New South Wales since the establishment of routine biotoxin monitoring in 2005. The intensity and duration of the 2016 *A. pacificum* bloom were unusual given the relatively low abundances of *A. pacificum* in estuarine and coastal waters of the region found in the prior 10 years.

2.2 Introduction

One of the most common and pervasive toxins produced by microalgae is saxitoxin (STX) and its analogs, also known as paralytic shellfish toxins (PSTs), which cause paralytic shellfish poisoning (PSP), a potentially fatal human illness (Ajani, Harwood & Murray 2017; Kao 1993). PSP associated symptoms include tingling and numbness in the lips, tongue, fingers and toes, muscular weakness and breathing difficulty (Ajani, Harwood & Murray 2017; Deeds et al. 2008). Death can occur due to respiratory failure in extreme cases (Deeds et al. 2008). These neurotoxins are produced by species of the genus *Alexandrium* Halim, and a single species of Gymnodinium Stein and one of Pyrodinium Plate in temperate coastal waters (Ajani, Harwood & Murray 2017; Landsberg et al. 2006; Oshima, Blackburn & Hallegraeff 1993; Usup, Kulis & Anderson 1994). PSTs are highly potent, such that only 100 to 200 cells $L{-1}$ in marine waters are sufficient to trigger uptake into shellfish above regulatory limits (Anderson 1997; Townsend, Pettigrew & Thomas 2001).

Species of *Alexandrium* are widely distributed and can form blooms in subarctic, temperate, tropical and subtropical regions (Taylor et al. 2003). *Alexandrium* species are distinguished from one another by morphological features such as cell size, differences in shape and ornamentation of the thecal plates, the presence or absence of a ventral pore and their chain forming capacity (Balech 1995). Some morphological characteristics, such as the presence of a ventral pore and the position of the anterior attachment pore are now considered homoplastic (Leaw et al. 2005), whereas other morphological characters such as the shape of the posterior sulcal plates, are considered to be very consistent and of taxonomic value (Balech 1995). About 33 species of *Alexandrium* have been recorded worldwide, of which around 10 species can potentially produce PSTs: *A. affine* (Inoue et Fukuyo) Balech; *A. andersonii* Balech; *A. pacificum* Litaker (= *A. catenella* Group IV ribotype); *A. australiense* Sh.Murray (= *A. tamarense* Group V ribotype); *A. minutum* Halim, *A. ostenfeldii* (Paulsen) Balech et Tangen; *A. catenella* (Whedon et Kofoid) Balech; *A. tamiyavanichii* Balech; and *A. taylori* Balech (Anderson, Cembella & Hallegraeff 2012; John et al. 2014; Tomas et al. 2012).

PSP was first reported in Australia in 1935, when typical PSP symptoms were observed following the consumption of wild mussels collected from Batemans Bay, New South Wales (NSW) (Le Messurier 1935). In 1986, the first PSP outbreak in Australia was recorded in Port

Philip Bay, Victoria, with *A. pacificum* (as *A. catenella*) as the causative organism (Ajani, Hallegraeff & Pritchard 2001; Hallegraeff 1992). Hallegraeff et al 1991, reported the presence of *A. pacificum* (as A. catenella) for the first time in a phytoplankton sample from Batemans Bay, NSW. In 1989, cysts of *Alexandrium* spp. were estimated to be in excess of 300 million cells L^{-1} in the ballast water at the port of Eden in southern NSW (Farrell et al. 2013; Hallegraeff 1992). To date, 11 species of Alexandrium have been identified in the south-eastern waters of Australia: *A. catenella* (Group 1)*, *A. affine**, *A. pacificum* (Group IV)*, *A. diversaporum* Sh.Murray et al., *A. fraterculus* (Balech) Balech, *A. margalefii* Balech, *A. minutum**, *A. ostenfeldii**, *A. pseudogonyaulax* (Biecheler) Horiguchi ex Yuki et Fukuyo, and *A. australiense* (Group V)* (Ajani et al. 2013; Anderson, Cembella & Hallegraeff 2012; Farrell et al. 2013; Hallegraeff et al. 1991; Hallegraeff et al. 2010; John et al. 2014; Murray et al. 2014; Murray et al. 2012) (species marked with * are PST producing). Between 2005 and 2012, *A. pacificum* was responsible for more than 50% of algal bloom related shellfish harvest closures in NSW; however, none of these incidents were associated with PST in shellfish tissue above the regulatory limit (Farrell et al. 2013). *Gymnodinium catenatum* Graham is also present in the NSW region; however, prior to 2019, this species was not associated with PST events in shellfish harvest areas. During April 2019, a positive PST result was associated with up to 7400 cellsL^{-1} of *G. catenatum* in a Nambucca River harvest areas (NSW FA, unpublished data).

Seafood industries make a significant contribution to the Australian economy, as they harvest about 227,000 tons of seafood annually, with a value of ~$AUD 2.2 billion (Ajani, Harwood & Murray 2017). The farm-gate value of commercial shellfish aquaculture in New South Wales (NSW) was estimated at $AUD 54.5 million during 2017/2018. This industry has steadily increased in value since 2014/2015 (~$AUD 41 million) (NSWDPI. 2019). The largest toxic dinoflagellate bloom event in Australia occurred in 2012, when a shipment of blue mussels (*Mytilus galloprovincialis* Lamarck, 1819) from the east coast of Tasmania was found to contain PSTs above the regulatory limit by Japanese import authorities (Campbell et al. 2013). This toxic event cost the Australian industry AUD ~$23 M in lost revenue. *Alexandrium catenella* (Group 1 genotype) was identified as the source of the toxins in this region (Campbell et al., 2013). Significant levels of PST were also recorded in scallops, clams, and rock lobsters with a resulting six-month harvest closure along 350 km of the Tasmanian coastline (Campbell et al., 2013).

In 2016, 2017 and 2018, *Alexandrium pacificum* was reported in the blue mussel (*Mytilus galloprovincialis*) shellfish harvest areas in Twofold Bay, NSW, an oceanic embayment on the south coast of NSW. The elevated concentrations of these species were associated with the first occurrence of PSTs above the regulatory limit since the establishment of the NSW Shellfish Quality Assurance Program in 2005. The identity of the species, its toxicity and the environmental conditions at the time of this shellfish contamination are examined in this study.

2.3 Materials and methods

2.3.1 Water and Shellfish Sampling

Two shellfish “harvest areas” have been designated within Twofold Bay, NSW (Figure 2.1 A,B). Three water sample sites (designated as 1, 3 and 4, Figure 2.1) for phytoplankton identification and enumeration and two shellfish sample sites (one for each of the harvest zones, A and B, at aquaculture leases proximate to water sites 1 and 4, respectively, Figure 2.1) for biotoxin testing were established in relation to these harvest areas in Twofold Bay in accordance with the NSW Marine Biotoxin Management Plan (MBMP) and the Australian Shellfish Quality Assurance Program (ASQAP) Operation Manual (NSWFA 2015, ASQAAC 2016). During the harvest season, fortnightly phytoplankton and monthly biotoxin samplings were conducted at these sites (NSWFA. 2015) (Table S1). When phytoplankton cell concentrations were found to be above the specified phytoplankton action limits (PAL), and/or a report of a positive biotoxin test result occurred, the sampling frequency was increased to weekly and the initiatives like harvest area closure is taken if the cell count of species of Alexandrium exceeds the limit (NSWFA 2015) (Table S2). For species of Alexandrium, a biotoxin test is triggered at 200 cells L^{-1}, while a closure occurs at 500 cells L^{-1} (NSWFA 2015).

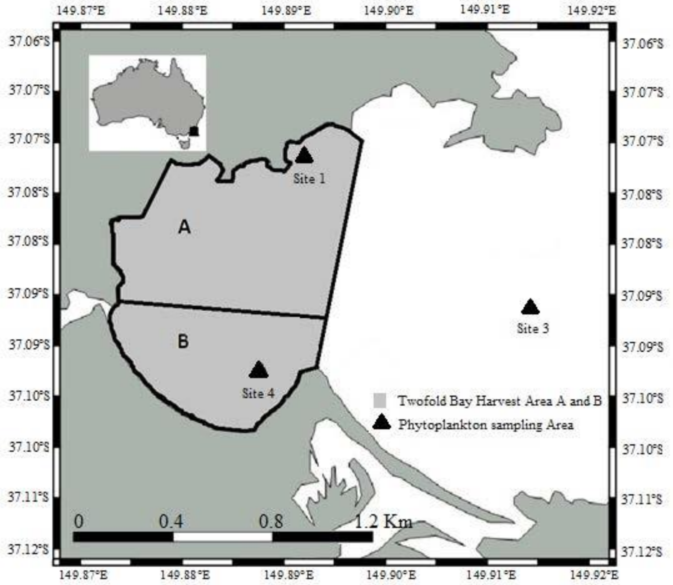


Figure 2.1. Map of Twofold Bay, New South Wales, Australia, showing sampling sites and aquaculture harvest areas A and B.

For phytoplankton identification and enumeration, 500 mL water samples were collected from a depth of 0.5 m from each site and preserved with Lugol's iodine solution. On 24 October 2016, 500 mL of seawater was collected from the same sites for molecular characterisation (Table 2.1). Vertical phytoplankton net haul (20 µm mesh) samples were also collected to support species identification, culture establishment and detailed morphological and molecular investigations.

Table 2.1. Morphological, molecular, toxicological and water mass characterization collected for Alexandrium pacificum bloom identification in Twofold Bay during the 2016 and 2018 sampling periods.

Alexandrium pacificum bloom identification in Twofold Bay 2016-2018, including water mass characterization for the 2016 bloom event.

Data collected	2016	2017	2018
Morphological			
Cell abundance	x	x	x
Strain isolation			x
Light microscopy	x	x	x
Scanning electron microscopy			x
Molecular			
LSU gene			x
SSU gene			x
qPCR	x		
Amplicon Sequencing	x		
Toxicological			
Strain toxicity LCMS/MS			x
Mussel biotoxin test	x	x	x
Oceanographic conditions			
Water mass characteristics	x		
Physico-chemical parameters	x		
Nutrients	x		

A combination of morphological, molecular, and toxicological data was collected from *Alexandrium pacificum* cultures, as well seawater, shellfish samples, and water mass characteristics across the 2016 and 2018 sampling periods (Table 2.1). For 2017, only cell abundance, light microscopy and mussel biotoxin testing were carried out.

2.3.2. *Alexandrium* Isolation and Culture Maintenance

Two nonaxenic monoclonal cultures (TFB_C/18 and TFB_G/18) were established from a net haul sample collected on 2nd August 2018. Single cell isolation of a species of *Alexandrium* was performed using drawn out glass pipettes (Pasteur pipettes) and a Nikon Eclipse TS100 inverted microscope (100× magnification). Isolated cells were transferred into Falcon®24 well culture plates containing 1 mL of five-times diluted K medium (Keller et al. 1987) without sodium silicate. Germanium dioxide was added at a concentration of 5 µg/mL to prevent diatom growth. Well plates were kept at 18 °C under a photon flux of 60–100 µmol photon m^{-2} s^{-1} on a 12/12 h dark/light cycle (white fluorescent tubes) and checked every alternate day. After three weeks, monoclonal cultures were transferred into 20 mL of K medium in a 70 mL gamma sterile polystyrene container with polyethylene caps (Thermo Fisher Scientific, Australia, Pty., Scoresby, Vic, Australia) and grown under the same conditions outlined above. One millilitre of culture from each strain was transferred into fresh medium every three weeks to maintain healthy growing cultures.

2.3.3. Species Identification and Enumeration

Phytoplankton samples preserved with acidic Lugol's iodide solution were concentrated by gravity-assisted membrane filtration. At first, the original 500 mL of the sample was mixed thoroughly and 300 mL was measured into a membrane filtration apparatus. The 300 mL of the sample was concentrated to 3.0 mL using gravity-assisted 5 µm membrane filtration (100× concentration). One millilitre of the concentrated sample was placed into a Sedgewick Rafter counting chamber. A minimum of 200 µL (1/5 of the entire chamber) was counted using a Zeiss Axiolab light microscope equipped with phase contrast (maximum magnification 400×) to provide an estimate of cell numbers. The threshold from this count is 50 cells/L. For examination of thecal plates, Lugol's fixed cells were stained with Calcofluor White and examined with epifluorescence.

Light microscopy (LM) of living and fixed cultured cells was done with a Leica DMRB (Leica Microsystems GmbH) equipped with differential interference contrast and epifluorescence optics at 400 and 640 times magnification with oil immersion objectives. Digital photos were taken using a Leica DFC420C camera (Leica Microsystems GmbH). For thecal plate visualization, Lugol-fixed cells were stained with Solophenyl Flavine 7GFE 500 (Ciba Speciality Chemicals, High Point, NC, USA) as described by Chomérat et al. (2017) and examined using epifluorescence(Chomérat et al. 2017).

For scanning electron microscopy (SEM), Lugol's fixed cells from the established cultures (isolated on 2 August 2018) were placed on a 5 µm Millipore filter (Merck Millipore Darmstadt, Darmstadt, Germany), rinsed in distilled water, and dehydrated in a series of increasing ethanol concentrations (30%, 50%, 70%, 85%, 90%, and 100%), followed by chemical drying with hexamethyldisilazane at room temperature. When completely dry, the sample was mounted on a stub and sputter coated with gold-palladium (Bal-Tec SCD 050; BAL-TEC Präparationsgerätevertrieb, Wallof, Germany). Cells were observed using a Tescan VEGA3 microscope (Elekronen-Optik-Service GmbH, Dortmund, Germany) at 10 kV.

2.3.4. Molecular Characterisation

2.3.4.1. Amplicon Sequencing and qPCR

Seawater samples collected on 24 November 2016 (Table 2.1) were filtered using 5 µm nitrocellulose filter (Merck Millipore) to concentrate the phytoplankton and stored at −80 °C prior to DNA extraction. DNA was extracted from the filter using MPBio FastDNA® SPIN kit for Soil according to manufacturer's protocol. DNA quality and quantity were examined using a Nanodrop (ND-1000, Thermo Scientific, Woltham, MA, USA). 18S amplicon sequencing of the V4 region was subsequently performed on the Illumina MiSeq 2 × 250 bp (bare pair) PE (paired end) standard sequencing platform (Ramaciotti Centre for Genomics, UNSW).

qPCR analysis was carried out using assays specific to *A. catenella, A. pacificum*, and *A. australiense* (Ruvindy et al. 2018). Species-specific primers were used to identify the species present in the sample (Table 2.2). The qPCR reactions were performed on a StepOne Plus Real-Time PCR System (Thermofisher Scientific, Waltham, MA, USA) platform with the following cycles: 95 °C for 10 s and 35 replicates of 95 °C for 15 s and 60 °C for 30 s. Melting curve analysis was performed at the end of each cycle to confirm amplification specificity by increasing the temperature to 95 °C for 10 s, then to 65 °C for 5 s, and 95 °C with ramp temperature of 0.5° C sec−1. Each 20 µL reaction contained 10 µL of SybrSelect™ Mix (ThermoFisher Scientific, Waltham, Massachusetts, USA), 0.5 µM of each primer, 1 µL template DNA, and 7 µL PCR-grade water. Standard curves used to quantify the cell concentrations were developed using a two known strains CS300 (*A. pacificum*) and AT-YC-H (*A. australiense*) grown in 18 °C, 12 h/12 h light cycle, and GSe medium (Blackburn et al. 2001).

Table 2.2. List of qPCR assays and primers used for *Alexandrium* species identification.

Species	Primers	
	Name	Sequence
A. catenella	ACT-US-408-F	5'-ACT TGA TTT GCT TGG TGG GAG-3'
	ACT-US-645-R	5'-AAG TCC AAG GAA GGA AGC ATC C-3'
A. pacificum	ACTA-416-F	5'-TCC TCA GTG AGA TTG TAG TG-3'
	ACTA-605-R	5'-GAC AAG GAC ACA AAC AAA TAC-3'
A. australiense	AusTv2-F	5'-CGG TGG GTG CAA TGA TTC-3'
	AusTv2-R	5'-GCA GGA AAA TTA CCA TTC AAG T-3'
	AusTv2-P	5' – CACAGGTAATCAAATGTCCACATAGAAACTG-3'

2.3.4.2. Bioinformatic Analysis of Amplicon Data

Bioinformatic analyses of the V4 region of 18S rDNA data were performed using QIIME 2 (v2019.1) (Bolyen et al. 2018). Six sets of raw sequence data were imported, summarized using DEMUX plugin, and filtered (where, Q = 20) for further processing. The DADA2 plugin was used for trimming, chimera detection, zOTU picking and mapping with the following parameters: –p-trim-left-f 0 –p-trunc-len-f 220 –p-trim-left-r 0 –p-trunc-len-r 220. Taxonomic classification was performed by comparing with a QIIME compatible database PR2 (Protist Ribosomal database) using QIIME 2′s feature-classifier plugin. The resulting files were exported and used for downstream analysis using R-3.6.1. The programs phyloseq (McMurdie & Holmes 2013), ggplot2 (Wickham 2016a), ape (Paradis, Claude & Strimmer 2004), qiime2R (Bisanz et al., 2018), tidyverse (Wickham 2016b) packages were used to create OTU table, taxonomic table, and bar plot to examine species taxonomic delineation and relative abundance.

2.3.4.3. DNA Extraction, PCR Amplification and DNA Sequencing

DNA was extracted from two established *Alexandrium* cultures (TFB_C/18 and TFB_G/18) cell pellets using the FastDNA spin kit for soil (MP Biomedicals, Solon, OH, USA) and the quality and the quantity of DNA was determined using a Nanodrop (Nanodrop2000; Thermo Scientific, Scoresby, Australia). Partial sequences of the rRNA genes large subunit (LSU) and small subunit (SSU) were amplified using previously published primers: D1F (Scholin et al., 1996), D3B (Nunn et al., 1996), SS3 and SS5 (Medline et al., 1988). All PCR reactions were performed in 25 µL reaction volumes containing 12.5 µL of 2X Immomix (Bioline, Sydney, Australia), 7.5 pmol of each primer, 1 µg µL−1 of BSA (Biolabs, Arundel, Australia), 1 µL of template DNA and 8.5 µL of PCR-grade water. The thermocycling conditions consisted of an initial denaturation step of 94 °C for 2 min, followed by 35 cycles of 94 °C for 20 s, 56 °C for 30 s, and 72 °C for 1 min, followed by a final extension step of 7 min. PCR products were checked by agarose gel electrophoresis. PCR products were purified using Zymoclean™ gel DNA recovery kit (Zymo Research, Irvine, CA, USA) and sent to Macrogen (Seoul, Korea) for sequencing.

2.4.4. Sequence Analysis and Phylogenetic Reconstruction

Sequences generated in this study were aligned with *Alexandrium* spp. data obtained from the GenBank reference database. The alignment algorithm ClustalW (Larkin et al. 2007) was used through the Geneious software (v 9.1.2) (Kearse et al. 2012) and manual inspection. Alignments were truncated to the same length. Phylogenetic trees were calculated using both a maximum likelihood (ML) and Bayesian Inference (BI) approach via Mr. Bayes 3.2.2 (Ronquist et al., 2012). The ML tree was inferred using PHYML (Guindon et al. 2010) with 1000 bootstraps (BS). The GTR (general time reversible) model with estimated gamma distribution was used for both analyses. BI was used to estimate the posterior probability (PP) distribution with Markov Chain Monte Carlo (MCMC) algorithm. Four heated chains with a temperature set at 0.2 were run simultaneously for 2,000,000 generations, a subsampling frequency of 1000, and a burn in length of 1000.

2.5. Biotoxin Analysis

2.5.1. Biotoxin Testing of Shellfish (Mussel) Samples from Twofold Bay

During 2016, 2017 and 2018, mussel samples (consisting of 12–14 individuals, minimum 100 g tissue) were taken from sampling sites (Figure 1) when *Alexandrium* cell numbers exceeded the regulatory limit of 200 cells L^{-1} (Table 2.1). Samples were frozen and couriered on ice to Symbio Laboratories, Sydney for the analysis of paralytic shellfish toxins (PSTs), amnesic shellfish toxins (ASTs) and diarrhetic shellfish toxins (DSTs), as per the requirements in the MBMP.

At the National Association of Testing Authorities (NATA) accredited commercial laboratory, PST analysis was by high performance liquid chromatography (HPLC) (Lawrence, Niedzwiadek & Menard 2005). Initial screening for PSTs included the analogues STX, GTX2, 3, C1, 2, GTX5, NEO, dcNEO, and GTX1, 4. If a positive result was reported, precolumn oxidation was used to confirm concentrations of STX, GTX2, 3, C1, 2, GTX5, dcSTX, dcGTX2, 3, NEO, dcNEO, GTX1,4, C3,4. AST (domoic acid (DA)), and DSTs (OA, dinophysistoxin 1 (DTX-1), dinophysistoxin 2 (DTX-2)), and pectenotoxin 2 (PTX-2) were analysed by liquid chromatography tandem mass spectrometry (LC-MS/MS) (Top et al., 2011, Villar-González et al., 2011). The lipophilic toxins cylindrospermopsin, gymnodimine, spirolide 1, azaspiracid 1, azaspiracid 2, azaspiracid 3, and yessotoxin were also included as part of this screening (Villar-González et al., 2011). Positive toxin results were reported as equivalent to ≥1.00 mg/kg DA (AST), ≥0.25 mg/kg OA equivalents (DSTs) and ≥0.10 mg/kg STX equivalents (PSTs).

In brief, about 5 g of shellfish flesh was used for the toxin analyses by Lawrence method (Lawrence, Niedzwiadek & Menard 2005). The flesh was homogenized using 3 mL of 1% acetic acid. Then, this mixture was boiled for 20 min in the water bath. This sample was allowed to cool down and the centrifugation was performed for 10 min at 3600× g. The supernatant was collected and the remaining pellet was mixed with 3 mL of 1% acetic acid. This prepared sample was centrifuged to separate the supernatant. These supernatants were mixed with water to get a final volume of 10 mL. A SPE C18 cartridge was used to perform the clean-up of this mixture. Oxidation of standards, PST positive reference matrix and sample was oxidised with a matrix modifier. After periodate oxidation of this sample, screening was performed, which was followed by the confirmation analyses.

2.5.2. Toxin Profile Determination of *Alexandrium* Culture

Approximately 500 mL of each *Alexandrium* culture of the two established monoclonal cultures (~1.39 × 10^6 cells of the strain TFB_C/18 and ~1.27 × 10^6 cells of the TFB_G/18 respectively) were centrifuged, and the resulting cell pellets were immediately frozen at −80 °C until further processing. Pellets were then transported to the Sydney Institute of Marine Science (SIMS), NSW, Australia, where they were tested for the presence of STX and 13 analogues (C1, C2, GTX2, GTX1, dcGTX2, GTX3, GTX4, dcGTX3, GTX5, GTX6, dcNEO, dcSTX, NEO) by LCMS.

Briefly, cell pellets were extracted using the method of Harwood et al. (2013)(Harwood et al. 2013). Five millilitres of 1 mM acetic was then added to each sample tube and vortexed for 90 secs. The sample tube was placed into boiling water bath for 10 min, cooled at room temperature, then placed in an ultrasonic bath for 1 min and finally centrifuged for 5 min. The supernatant was then used either with or without dilution for chromatographic separation on a Thermo Scientific™ ACCELA™ UPLC system (Table S4).

A Thermo Scientific™ Q EXACTIVE™ high resolution mass-spectrometer equipped with an electrospray ionization source was used for the analysis. The following source parameters were used in all experiments: a capillary temperature of 263 °C, a spray voltage of 3.5 kV, an auxiliary gas heater temperature of 425 °C, a sheath gas and an auxiliary gas flow rate of 50 and 13 (arbitrary units). The mass spectrometer was operated in polarity switching mode scanning across the range of m/z 100–500. LC-MS was performed on a Thermo Scientific™ ACCELA™ UPLC system by the method published by Boundy et al. (2015). Separation was carried out by an Acquity UPLC BEH Amide 130 (150 mm × 2.1 mm i.d., 1.7 µm particle size) column. Mobile phases were A. Water: Formic acid: NH4OH (500:0.075:0.3) and B. Acetonitrile:Water:Formic Acid (700:300:0.1). The injected volume was 5 µL. Thermo Xcalibur software (version 3.0.63, Thermo Fisher Scientific, Inc.) was used for the data analysis. Analytical standards for all of the PST analogues were obtained from National Research Council, Canada.

2.6. Oceanographic and Water Mass Characteristics

Prior to, and during the 2016 bloom event, water quality and oceanographic data were collected fortnightly at six locations in the bay (Figure 2.1) as part of a routine monitoring program in Twofold Bay (NSW DPI Lands, 2016). Field parameters including water column depth (m), dissolved oxygen (%, mg/L), turbidity (ntu), pH (pH units), salinity (ppt), conductivity (µS/cm) and temperature (°C) were measured using a YSI 6600 V2-4 WQ sonde to a maximum depth of 50 m. Additionally, a secchi disc was used to measure water clarity at each site. Laboratory parameters including suspended solids (mg/L), turbidity (ntu), ammonia as N (mg/L), ammonium (NH4+) 6 (mg/L), total nitrogen as N (mg/L), total phosphorus as P (mg/L) and chlorophyll a (µg/L) were measured from water samples collected using a vertical oriented Kemmerer sampler made of polycarbonate (with silicone end caps) and dropped to depths of ~1 m & ~8 m for sites W1, W2 and Bay 1; ~1 m & ~5 m for site CB1; and ~1 m & ~16 m for site Bay2. All water samples were kept on ice and immediately transported to a commercial laboratory for analysis. Furthermore, throughout the 2016 sampling campaign, oceanographic conditions were monitored using sea surface temperature data and eastward geostrophic current velocity data for each day sampled (Available online: http://oceancurrent.imos.org.au/product.php).

2.4 Results

2.4.1 Initial Light Microscope Identification and Cell Abundances (2016–2018)

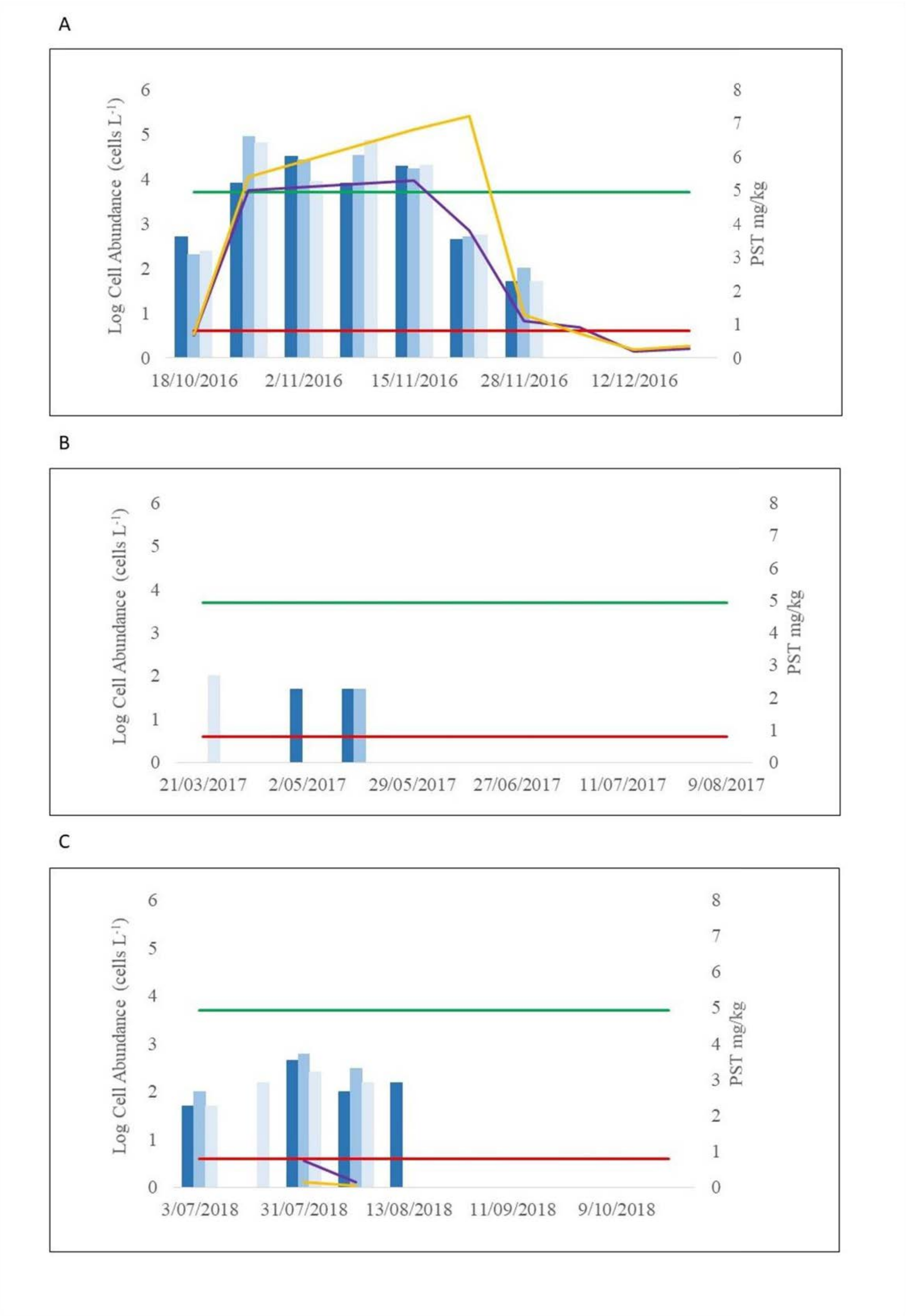


Figure 2.2. *Alexandrium pacificum* cell abundance at three sites (Site 1 dark blue; site 3 blue and site 4 light blue) across (A) 2016; (B) 2017; and (C) 2018 bloom events. Dark purple line is paralytic shellfish toxin (PST) concentration in mussels (mg/kg) collected from Area A; yellow line is PST concentration in mussels (mg/kg) from Area B; green line is DPI Phytoplankton Alert Level (5000 cells L^{-1}) and red line is DPI Regulatory Limit for PST in shellfish (mg/kg) in shellfish.

In a phytoplankton sample collected on 18 October 2016, cells were identified as *Alexandrium pacificum* and enumerated to 500 cells L^{-1} at site 1 within Twofold Bay (Figure 2.2). The concentration increased to 89,000 cellsL^{-1} during the next week (Figure 2.2). While the highest cell concentrations of Alexandrium were reported from phytoplankton sampling sites in Twofold Bay, evidence of the bloom was detected in other south coast harvest areas (NSWFA 2017). Along the coastal shelf there were elevated concentrations (1200–15,000 cells L−1) of "*Alexandrium pacificum*" detected up to 13 km north and 21 km south of Twofold Bay. During the bloom event, PST levels did not exceed the regulatory limit in other NSW shellfish harvest areas. Maximum cell concentrations reported were 89,000 cells L^{-1}, 50 cells L^{-1}, and 600 cells L^{-1} during 2016, 2017, and 2018, respectively (Figure 2.2).

2.4.2 Mussel Toxin Analysis (2016–2018)

Positive PST results were reported in Twofold Bay during 2016, 2017 and 2018. A mussel tissue sample collected on 18 October 2016 was positive for paralytic shellfish toxins (PSTs), with a maximum concentration of 0.78 mg/kg total PSTs. Additional samples collected 24 October 2016 showed that toxin levels had increased (maximum 5.4 mg/kg total PSTs) (Figure 2.2). The bloom continued and maximum concentration of PSTs reported was 7.2 mg/kg on 21 November 2016. (Figure 2.2) Maximum concentrations reported were 7.2, 0.22 and 0.74 mg/kg PST during 2016, 2017 and 2018, respectively (Figure 2.2).

2.4.3. Amplicon Sequencing and qPCR (2016)

As individual species of the former *Alexandrium tamarense* species complex can be highly morphologically similar by light microscopy (John et al 2014), molecular genetic methods were used to confirm identification. The analysis of amplicon sequencing data from the 24 November 2016 samples showed that of all the classified OTUs, dinoflagellates were the most abundant phylum with an average abundance of 95.05%, followed by Metazoa (1.53%) and Ciliophora (1.50%) (Figure 2.3A). Other phyla made up less than 2%. At the genus level, the most commonly observed genus was *Alexandrium* with an average abundance of 94.15% (Figure 2.3B) (Table S3).

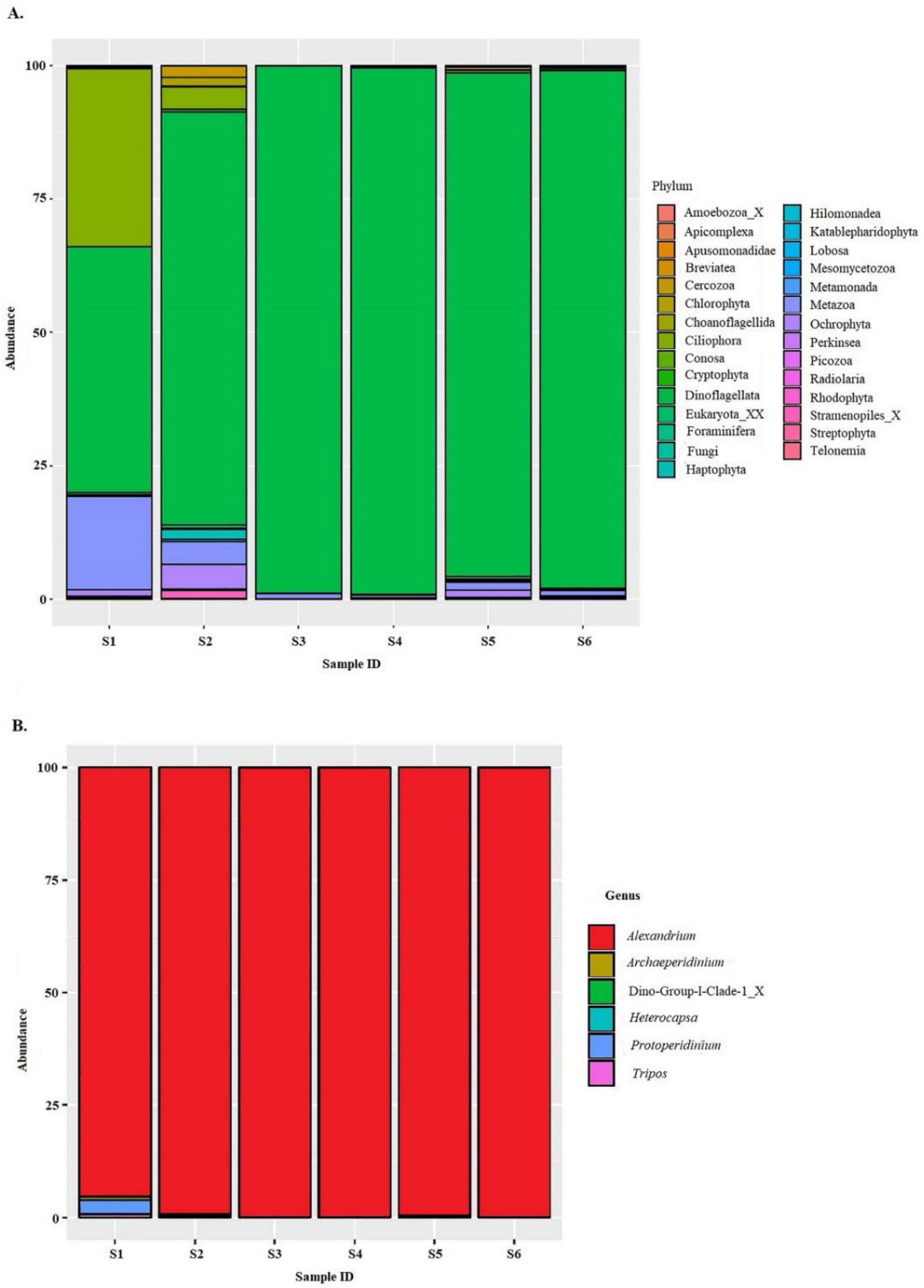


Figure 2.3. Eukaryotic community composition in samples. (A) Abundances of the dominant group in the phylum level. (B) Abundances of the dominant genus of the most dominant phylum, dinoflagellata S1 and S2 stand for sample from the sampling site 1 (Figure 2.1), S3 and S4 stand for sample from the sampling site 3 (Figure 2.1), S5 and S6 stand for sample from the sampling site 4 (Figure 2.1).

Finally, the phylogenetic analysis of the *Alexandrium* obtained revealed that the sequences were placed in a strongly supported clade with sequences from multiple *Alexandrium pacificum* strains from the NCBI database (Figure 2.4, 66.3/0.71 ML BS/Bayesian PP).

Figure 2.4. Phylogeny of the amplicon sequences of *Alexandrium*. The trees were constructed with Bayesian Inferences (MrBayes). Numbers at nodes represent posterior probabilities from Bayesian Inferences (BI) and bootstrap support values from Maximum Likelihood (ML) based on 1000 replicates.

Molecular identification using qPCR assays specific to *A. pacificum*, *A. catenella*, and *A. australiense* was performed for 24 November 2016. All samples were dominated by *A. pacificum.* The species *A. catenella* was absent in all three tested leases, whereas *A. australiense* was present in the water sample site 1 at a very low concentration of 1 cells L^{-1}. The cell density variation across the sample sites was high, ranging from 345 cells L^{-1} in water sampling site 1, 12,148 cells L^{-1} in water sampling site 3 and up to 13,456 cells L^{-1} in the water sampling site 4 (Figure 2.1)

2.4.4. Morphological and Phylogenetic Analysis of Cultured Strains

Detailed light microscopy (LM, Figure 2.5) and scanning electron microscopy (SEM, Figure 2.6) analysis, was conducted on the two cultured monoclonal strains isolated on 2nd August 2018. The two strains conformed to the species description of *Alexandrium pacificum* (Figure 2.5 and Figure 2.6). Cells occurred mainly as single cells, but were also observed to form chains in culture (up to 12 cells). Cells were approximately 35 ± 3.1 µm wide (n = 15) and 36.2 ± 3 µm long (n = 15), contained elongated chloroplasts and a median-dorsal, sausage-shaped nucleus (Figure 2.5A–D). The episome was helmet-shaped and the hyposome roughly trapezoidal with a slightly longer left posterior side (Figure 2.5E). The cingulum was descending about one cingulum height (Figure 2.5F–H). The observed plate formula was APC 4′ 6″ 6c 8s 5‴ 2⁗ (Figure 2.5F–O and Figure 2.6A–K). The first apical plate (1′) was rhomboidal with longer anterior right and posterior left sides of variable relative lengths (Figure 2.5F–H). Plate 1′ contacted the Po and Sa plates (Figure 5F–J,N). In some cells, a ventral pore was present (Figure 2.5I,J,O and Figure 2.6B arrows). The sixth precingular plate (6″) was not triangular, an asymmetrical trapezoid shape and about as high as wide or slightly wider (Figure 2.5F–I,N and Figure 2.6C). The second antapical plate was transversely extended (Figure 2.6G,I). Eight sulcal plates were documented (Figure 2.5L,M and Figure 2.6K). The posterior sulcal plate (Sp) was symmetrical, pentagonal, and longer than wide (Figure 2.5I,L,M and Figure 2.6I), reached the antapex (Figure 2.6G–I), and sometimes had a posterior connecting pore (Figure 2.6J,K arrows). Sulcal plates were moderately developed (Figure 2.6F–J). Thecal plates were smooth with scattered pores of two size classes and distributed pairs of pores (often a smaller and a larger pore in close contact) (Figure 2.6L,M). Only very few apical pore complexes (APC) could be observed and none had an anterior connecting pore.

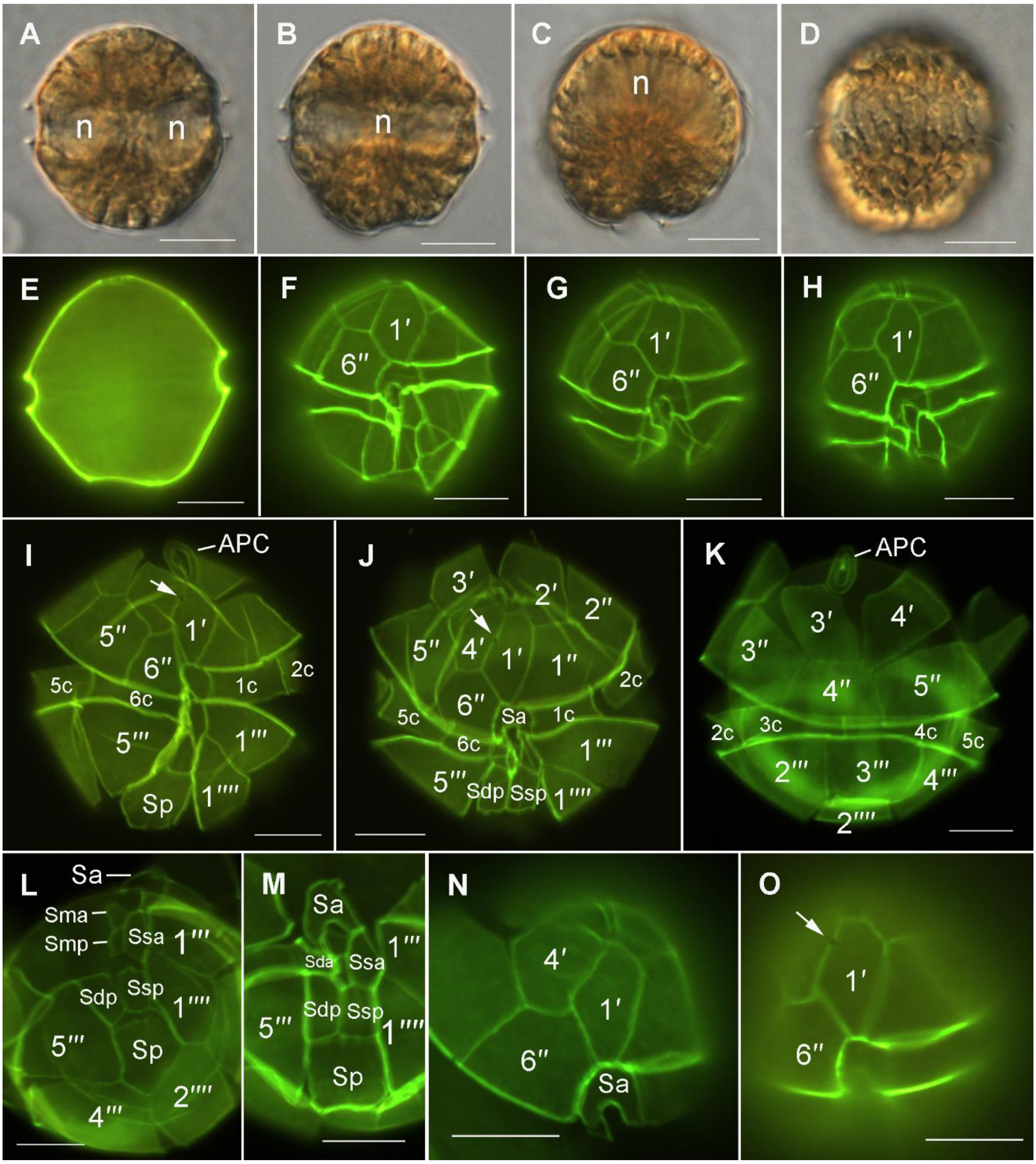


Figure 2.5. Light micrographs of *Alexandrium pacificum* using differential interference contrast (A–D) and epifluorescence microscopy (Solophenyl Flavine staining) of Lugol-fixed cultured cells (E–O). (A) Ventral view with focus in the cell middle, notice the two ends of the sausage-shaped nucleus (n). (B) Dorsal view with focus in the dorsal half of the cell showing the path of the nucleus (n). (C) Antapical view with focus in the cell middle, notice the sausage-shaped nucleus (n) dorsally. (D) Dorsal view with focus on the peripherally located, elongated chloroplasts. (E) General cell shape. (F–H) Ventral view showing the shapes of the first apical (1′) and sixth precingular (6″) plates. Note the different degree of asymmetry in 1′. (I,J) Ventral

views of squeezed thecae showing the thecal plates. (K) Doral view of a squeezed theca showing the thecal plates. (L) Ventral to antapical view showing sulcal plates. (M) Sulcal plates. (N) Ventral epithecal view showing the shapes of characteristic plates 1′, 6″, and anterior sulcal plate (Sa). (O) The first apical plate (1′) with ventral pore (arrow). Scale bars = 10 μm.

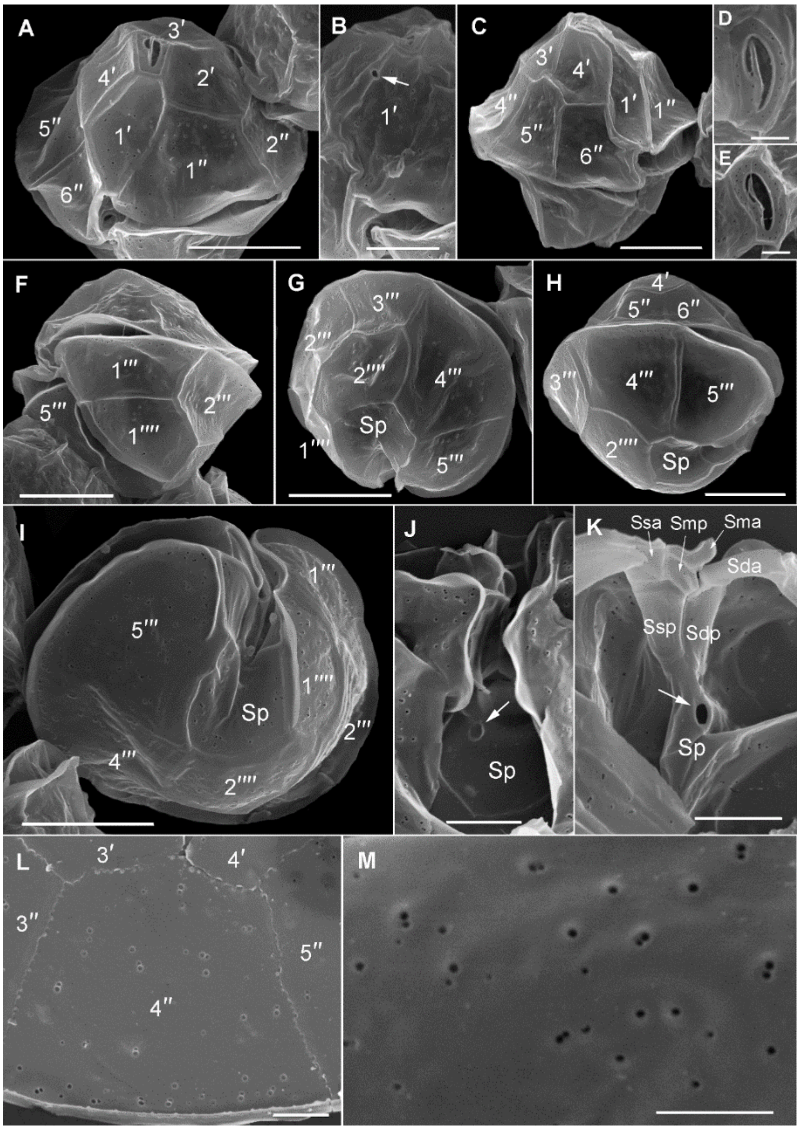
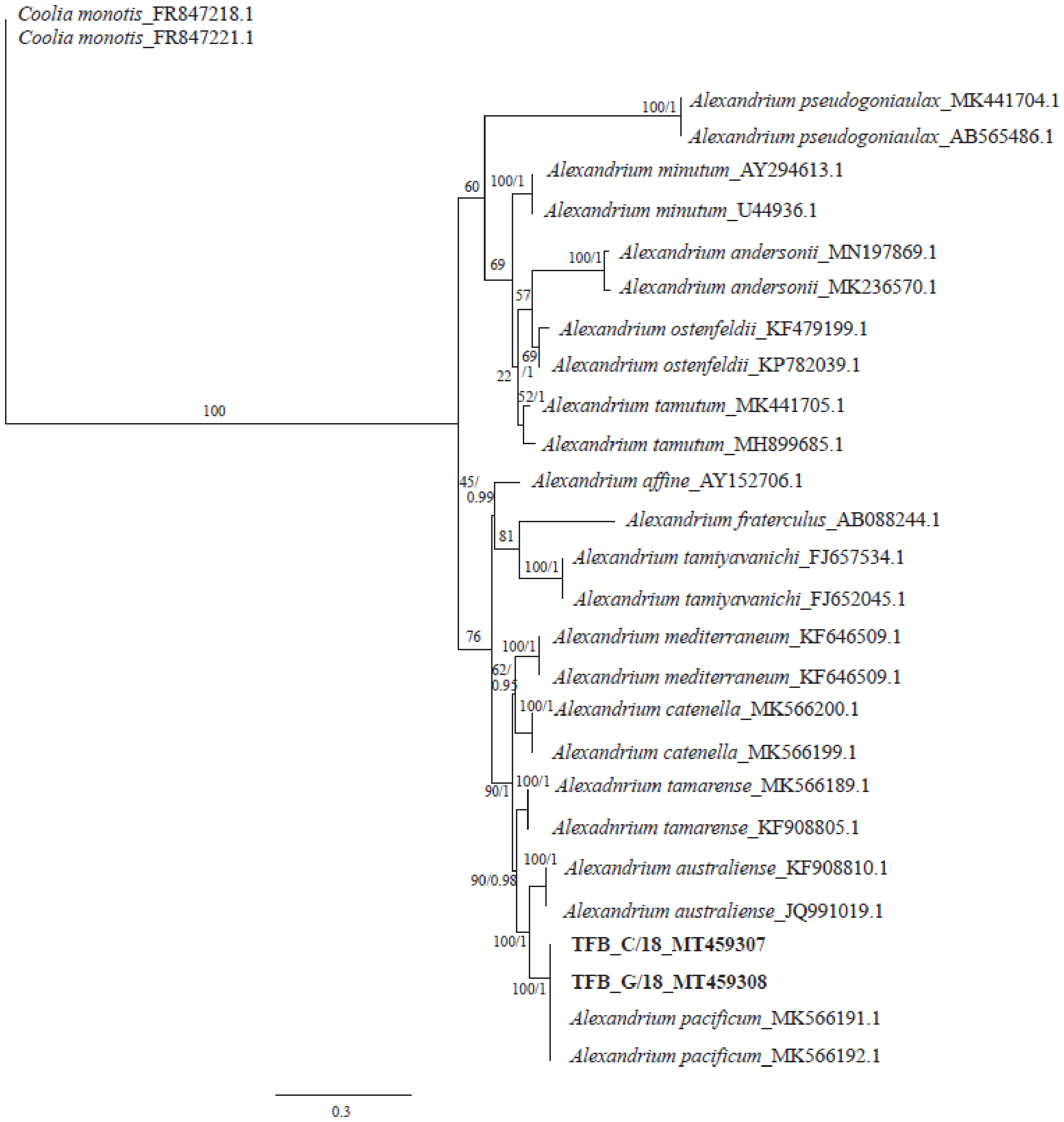


Figure 2.6. Scanning electron microscopy of *Alexandrium pacificum*. (A) Ventral of the epitheca. (B) Detail of a first apical plate (1′) with ventral pore (arrow). (C) Right lateral to ventral cell view. (D,E) Apical pore complex. (F) Left lateral view of a hypotheca. (G) Hypotheca in antapical view. (H) Right lateral cell view. (I) Hypotheca in antapical view showing the sulcus. (J) Detail of the posterior sulcal plate (Sp) with posterior connecting pore (arrow). (K) Inside view of the sulcal area of the hypotheca showing sulcal plate details. Note

the posterior connecting pore (arrow). (L,M) Thecal pore pattern and sizes. Scale bars = 10 µm, (B,J,K) 5 µm, (D,E,L,M) 2 µm.

The phylogenetic analysis of the two strains isolated on 2 August 2018, using Maximum likelihood and Bayesian Inference conducted on the SSU rRNA and LSU rRNA (D1/D3) regions showed that they clustered together with other *Alexandrium pacificum* strains with full support (Figure 2.7A,B, Bayesian PP = 1, ML BS = 100).



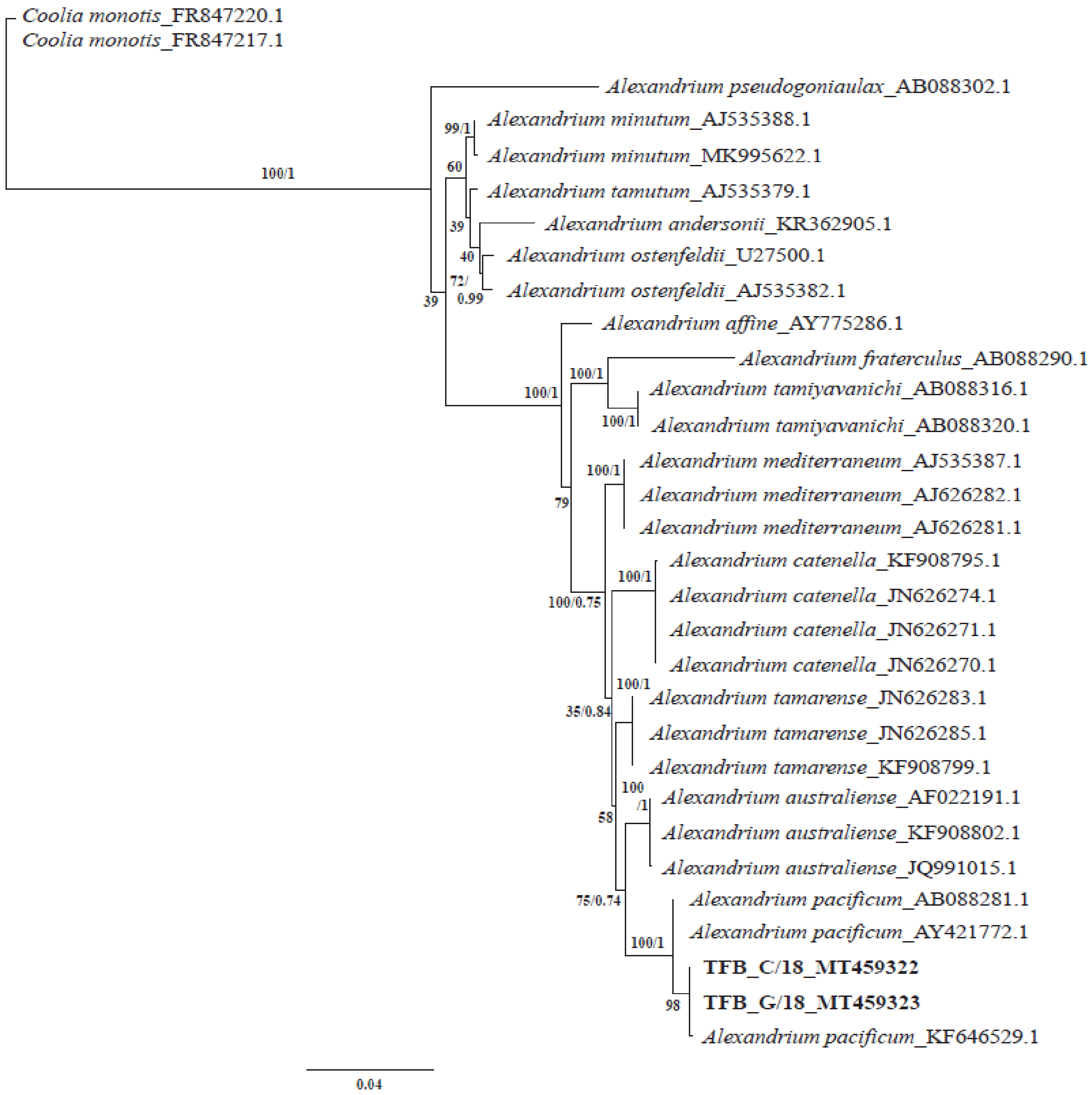


Figure 2.7. (A,B) Phylogeny of Alexandrium. The trees were constructed with Bayesian Inference (MrBayes). Numbers at nodes represent posterior probabilities from Bayesian Inferences (BI) and bootstrap support values from Maximum Likelihood (ML) based on 1000 replicates. (A) Phylogenetic tree based on large subunit (LSU) rRNA. (B) Phylogenetic tree based on small subunit (SSU) rRNA.

2.4.5. Toxin Profile of *Alexandrium pacificum* Strains

The results from the LC-MS for the PSTs confirmed that both of the strains (TFB_C/18 and TFB_G/18) isolated from Twofold Bay on 2 August 2018 (Table 2.1), TFB_C/18 and TFB_G/18, produced various PST analogues. Both of the strains produce the analogues C1, C2, GTX1, GTX4, GTX5, dcSTX and NEO in different concentrations. The strain TFB_C/18 can also produce two more analogues STX and dcNEO (Table 2.3).

Table 2.3. Toxin data of *Alexandrium pacificum*.

Target Compounds	Strain TFB_C/18		Strain TFB_G/18	
	Total Toxin (ng)	Toxin per cell (pg/cell)	Total Toxin (ng)	Toxin per cell (pg/cell)
C1	143.1	0.103	349.8	0.274
C2	1893.2	1.358	821.5	0.644
GTX2	ND	ND	ND	ND
GTX1	672.2	0.482	820.8	0.643
dcGTX2	ND	ND	ND	ND
GTX3	ND	ND	ND	ND
GTX4	692.1	0.496	728.2	0.571
dcGTX3	ND	ND	ND	ND
GTX5	1110.4	0.796	2132.6	1.671
GTX6	ND	ND	ND	ND
STX	83.9	0.0602	ND	ND
dcNEO	112.7	0.0808	ND	ND
dcSTX	157.2	0.113	235.8	0.185
NEO	816.3	0.585	370.4	0.29

2.4.6. Oceanographic and Water Mass Characteristics

A total of 96 water samples were collected from eight sampling times between 20 September 2016 to 17 January 2017, and water mass characteristics are summarised in Table 4. The most notable water characteristics preceding the bloom event was an increase in chlorophyll a (from a background minimum of 1 μg/L to peaking at 6 μg/L at a depth of 8 m on 4 Oct 2016), and a decrease in water column temperature during the bloom (a minimum temperature of 14.6 °C at a depth of 16 m on 1 Nov 2016 with a maximum over the study period reported as 22.7 °C). There was little difference in water column structure observed (salinity/temperature profiles) at the eight sampling times between 20 September 2016 and 17 January 2017.

Table 2.4. A summary of water quality measurements taken within Twofold Bay throughout the 2016 bloom sampling campaign. Data from each site has been combined and summarised to provide a general description of the water mass characteristics within the bay (*LOR = Limit of reporting).

A	DO	DO	Turbidity	Secchi Depth	pH	Salinity	Conductivity	Temperature	Suspended Solids
	%	mg/L	ntu	m		ppt	µS/cm	°C	mg/L
LOR	0.1	0.01	0.1	0.1	0.01	-	-	0.01	1
min	85.9	7.0	0.0	1.4	8.0	34.9	52973.0	14.6	1.0
max	112.6	9.0	7.4	16.0	8.2	35.9	54203.0	22.7	40.0
mean	100.9	7.9	1.0	4.8	8.1	35.5	53675.9	16.7	6.4

B	Turbidity	Ammonia	Ammonium	Total Nitrogen	Total Phosphorus	Chl a
	ntu	mg/L	mg/L	mg/L	mg/L	µg/L
LOR	0.1	0.02	0.02	0.05	0.005	1
min	0.2	0.020	0.019	0.1	0.005	1.0
max	3.2	0.1	0.1	0.6	0.015	6.0
mean	0.7	0.0	0.0	0.2	0.0	1.9

On 20 September 2016, a weak south-flowing current was observed in Twofold Bay. By 4 October 2016, a moderate to strong south-flowing current was reported. On 18 October 2016, a weak north-flowing current, influenced by an East Australian Current clockwise eddy located off continental shelf directly east of Twofold Bay persisted until mid-late October, when this eddy moved well north of Twofold Bay and directed warmer waters offshore. Inshore waters at this time were characterised by unusually cooler temperatures (Figure 2.8).

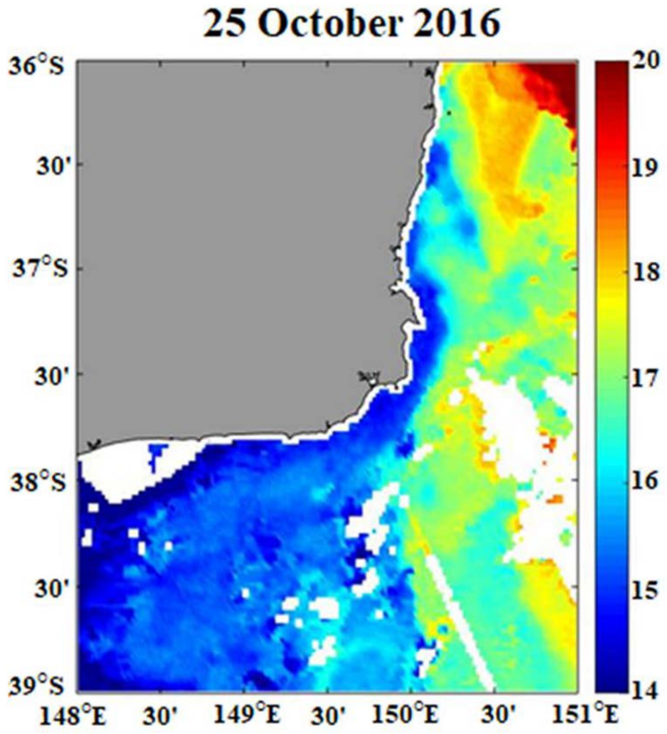


Figure 2.8. Three-day mean of sea surface temperature (SST) of the south-eastern Australian coastline on 24 October 2016 showing the warm sea-surface temperature extending from the north as red and the cold nutrient rich water extending from Bass Strait in the south as blue (Figure courtesy of Charitha Pattiaratchi, Australian National Facility for Ocean Gliders (ANFOG).

2.5. Discussion

2.5.1. The 2016–2018 *Alexandrium* Bloom Events

Since the initiation of the NSW Shellfish Quality Assurance Program in 2005, the majority of PST-positive events associated with *Alexandrium* have been reported from the NSW estuaries: Hawkesbury River; Georges River and Wagonga Inlet, with *Alexandrium pacificum* (=Group IV, formerly reported in Australia as "*A. catenella*") as the main causative agent of PSP toxicity (Ajani et al. 2013). In October 2016, high cell densities of this same species was detected in the coastal waters offshore of Twofold Bay, NSW, an unprecedented event for this location in south eastern Australia. The maximum cell density (89,000 cells L^{-1}) was also the highest cell count of this species ever reported in NSW, Australia, and led to approximately four months of shellfish harvest closures (NSWFA. 2017)(NSWFA 2017). Moreover, this bloom event was so extensive that A. pacificum cells were detected up to 13 km north and 21 km south along the adjacent coastline (NSWFA 2017). In 2017 and 2018, subsequent blooms of *A. pacificum* occurred in Twofold Bay. While significant harvest days were lost to the local shellfish industry during these blooms, no illnesses due to the consumption of shellfish in Twofold Bay during these events was reported.

Confirmed detections of algal biotoxins above the regulatory limit in NSW shellfish is relatively rare (Deeds et al., 2008) and prior to 2016, the maximum reported concentration of PSTs in NSW was 0.66 mg/kg, less than the harvest closure limit of 0.8 mg/kg PST equivalent. A PST concentration of ~10 mg/kg was measured during the unprecedented Tasmanian PST event which led to a worldwide product recall in 2012 (Bolch et al. 2014). That event was the first report of an *A. catenella* bloom in Australian waters and A. catenella continues to be the primary source of PSTs in Tasmanian water since then (Bolch et al 2014).

To get a further insight into the drivers of the 2016 unprecedented *Alexandrium pacificum* bloom in Twofold Bay, the eukaryotic microbial community and water mass data were examined. Though eukaryotic microbial interactions during *Alexandrium* blooms have rarely been studied, the link between the microbial community structure and the progression of a bloom has been reported, with evidence suggesting the growth of *Alexandrium sp.* succeeds the depletion of diatom growth (Zhou et al. 2018). During our study, the abundance of diatoms was very low during the bloom, with 94.15% of the eukaryotic microbial community comprised of *Alexandrium sp*. (Figure 2.3). While the available amplicon database for protists does not support the identification of *Alexandrium* to species level, we conducted phylogenetic analyses on the resultant OTUs in order to understand the *Alexandrium* species involved, with the resulting OTUs clustering with *A. pacificum* (Figure 2.4). Species specific qPCR also confirmed the abundance of Alexandrium pacificum during the 2016 bloom event. These results concur with *A. pacificum* (within the former "*Alexandrium tamarense* complex") as the most widespread *Alexandrium* species along the New South Wales and Victorian coastal areas (Ajani et al. 2013).

It is important to understand the environmental factors that significantly contribute to the initiation and termination of harmful algal blooms (HABs). These may include elevated nutrient levels, availability of micronutrients, water stratification, and seeding from benthic cysts during favourable conditions (Condie, Oliver & Hallegraeff 2019). The availability of inorganic nitrogen and inorganic phosphorus can shift the eukaryotic community composition in oceans from a dinoflagellate dominated community to one of diatoms (Zhou et al. 2018). The flow of unusually cool and nutrient rich water into Twofold Bay was observed during our study and may have contributed to the unprecedented growth of this taxa at this time. Furthermore, following the initial detection of *A. pacificum* in 2016, this organism was detected again in 2017 and 2018. The presence of *Alexandrium* cysts have already been reported in the major shipping port of Eden (Twofold Bay) (NSWFA 2017). As high abundances of *A. pacificum* have occurred repeatedly, it is possible that cyst beds have been established in this region, and that later blooms have occurred through reseeding of the area from an established cyst bed. High rainfall, low minimum air-temperatures and low wind speed influencing *Alexandrium catenella* (Group 1 genotype) blooms has been reported for eastern Tasmania (Bolch et al. 2014). Additional data collection would be required in follow-up studies in order to characterize these factors for *Alexandrium pacificum* blooms in New South Wales.

2.5.2. *Alexandrium pacificum* Species Identification

Species belong to the *Alexandrium tamarense* complex are cryptic and cannot be distinguished from one another using light microscopy alone (John et al 2014). Some aspects of the morphological appearance of *Alexandrium* cells from Twofold Bay initially resembled that which are commonly reported for *A. catenella* (= Group I, formerly known as *A. fundyense*), for example, they were commonly found as single cells rather than in short chains, a common feature of *A. pacificum* (NSWFA 2017). However, further investigation using qPCR, amplicon sequencing, and later, culture isolation and characterisation, correctly identified the causative organism as *Alexandrium pacificum* (Figure 2.3 and Figure 2.4), with *Alexandrium catenella* (=Group 1) not found to be present. Morphological characterisation of the cultured strains showed the typical *A. pacificum* morphology, such as the cell size and shape with its distinct plate structure (John et al 2014). Cells were slightly longer than wide and occurred either as single cells or in chain form, with helmet-shaped episome and roughly trapezoidal hyposome (John et al 2014). This study documented the rare formation of 12 cell chains, which is the first time this has been reported for *A. pacificum* to our knowledge. The strains usually have a smooth cell surface, ornamented with many scattered small pores (John et al 2014). In our study, we also observed different sized pores, also in close contact (pairs), which has not been reported before (Figure 2.6L,M). The plate formula is Po, 4′ 6″ 6c 8s 5‴ 2⁗, which is same as described for other *A. pacificum* strains. The irregularly rhomboidal first apical plate contacted the Po and Sa plates (Figure 2.5F–J,N). Moreover, we observed the presence of a ventral pore, which has not been reported for *A. pacificum* before (Figure 2.4B and Figure 2.5 I,J,O arrows). The number of sulcal plates may vary from 8 to 10 (John et al 2014) and in this study, we found the presence of eight sulcal plates (Figure 2.5L,M and Figure 2.6K). A pentagonal Sp plate with same length and width is commonly found in A. pacificum stains (John et al 2014), whereas in our study, we found the length is slightly longer than the width (Figure 2.5I,L,M and Figure 2.6I). A posterior connecting pore was also present in the Sp plates as previously reported (John et al 2014). Generally, an anterior connecting pore is found in the Po (John et al 2014). However, in our study, no anterior connecting pore was observed.

Phylogenetic analysis revealed that the isolated strains clustered in a well-supported clade with other *A. pacificum* (Group IV of the *A. tamarense* species complex) strains (Figure 2.7A,B). Both of the trees based on LSU and SSU sequences show those strains position with other A. pacificum in the same clade (Figure 2.7A,B).

Both clonal isolates from this study produced PSTs, with little variation found in their toxin profiles. *Alexandrium pacificum* strains produce C1, C2, GTX1, GTX4, GTX5, dcSTX, dcNEO, NEO and STX in different concentrations, which has been reported in previous studies of *A. pacificum*. Different STX analogues like B1, C1, C2, GTX 1-6, dcGTX3, dcSTX, dcNEO, NEO and STX have been reported in different studies of *A. pacificum* around the world (Hallegraeff et al. 1991; Harwood et al. 2013; John et al. 2014; Krock, Seguel & Cembella 2007; Murray et al. 2011; Negri et al. 2003; Orlova et al. 2007; Orr et al. 2013a; Sebastián et al. 2005). The toxin data presented in this study are indicative only, and future work should include a more rigorous investigation into the intra- and inter-strain toxin variability of A. pacificum blooms in southeastern Australia.

2.5 Conclusions

This study reports on the first contamination of blue mussels (*Mytilus galloprovincialis*) due to an unprecedented bloom of the toxic dinoflagellate *Alexandrium pacificum* in Twofold Bay, Australia. This was the first record of PSTs above the regulatory limit in commercial aquaculture in south-eastern Australia. Unequivocal identification of this species was undertaken using molecular genetic tools complemented with light and scanning electron microscopy.

This region of the world is a global hotspot for ocean warming (Suthers et al. 2011). Changes such as increasing water temperature, rainfall, salinity, and nutrient availability may influence the frequency, duration and extent of HABs in this region into the future (Condie, Oliver & Hallegraeff 2019). More studies are required to determine the specific factors which influence *A. pacificum* blooms in this region.

Chapter 3

Comparative *sxt* gene expression following exposure to the calanoid copepod (*Parvocalanus crassirostris*) in strains of *Alexandrium pacificum* with varying production of paralytic shellfish toxins

3.1 Abstract

The potent neurotoxin Paralytic Shellfish Toxins (PSTs) are known to be synthesized by several species of *Alexandrium, Gymnodinium catenatum,* and *Pyrodinium bahamense* in marine waters. Despite several studies being conducted to understand the regulation of PST biosynthesis in these organisms, the exact mechanisms of this remain unclear. In our study, we performed a study investigating differential gene expression of genes involved in PST biosynthesis (*sxt*) using a Nanostring gene array. In this study, we investigated whether the presence of water-borne cues from the locally common copepod *Parvocalanus crassirostris* may increase PST production and change the regulation of PST related genes in *Alexandrium pacificum* strains. We found that several analogues of the transcripts of *sxtA,* (*sxtA1* and *sxtA4*) were significantly more abundant in the PST-producing stains compared to the non-PST producing strains of Alexandrium pacificum. We found that the presence of *Parvocalanus crassirostris* could significantly increase PST production in the PST-producing strains, but that no significant upregulation of *sxt* genes was observed. This indicates that PST biosynthesis appears not be regulated at the transcriptional level in marine dinoflagellates. Further studies are required to identify whether *sxt* genes are regulated at the post-transcriptional or translational level in *Alexandrium pacificum*.

3.2 Introduction

Species of the genus *Alexandrium* are considered are widely studied due to their capacity to produce the potent neurotoxin, Paralytic Shellfish Toxins (PSTs), also known as saxitoxins (Anderson, Glibert & Burkholder 2002). Saxitoxin and its 57 analogs (STXs) are alkaloid toxins that can naturally be produced as secondary metabolites by freshwater cyanobacteria (prokaryotes) and marine dinoflagellates (eukaryotes) (Kellmann et al. 2010; Wiese et al. 2010). Accumulation of these toxins in the marine food web can lead to Paralytic Shellfish Poisoning (PSP) in humans and marine vertebrates by interacting with the voltage-gated sodium, potassium and calcium ion channels of the nervous system (Catterall 1985; Cestèle & Catterall 2000; Su et al. 2004; Wang, Salata & Bennett 2003).

The PSTs appeared to be synthesized through a similar biosynthetic pathway using the same precursors in both prokaryotic cyanobacteria and eukaryotic dinoflagellates (Hackett et al. 2004; Verma et al. 2019). The initial steps of the PST biosynthesis process have been proposed to be the same in the PST producing dinoflagellates and cyanobacteria (Cho et al. 2019; Tsuchiya et al. 2017; Verma et al. 2019). About 30 biosynthetic steps that are involved in this process have been identified from 6 cyanobacterial genera so far (Kellmann et al. 2008). Fourteen *sxt* genes (*sxt*A – *sxt*I, *sxt*P – *sxt*S and *sxt*U) have been identified in PST producing cyanobacteria and are considered as "core genes" (Kellmann et al. 2008; Murray et al. 2011). Among them, the direct involvement of eight of the core *sxt* genes (*sxtA, sxtB, sxtD, sxtG, sxtH* or *sxtT, sxtI, sxtS, sxtU*) have been reported (Kellmann et al. 2008). The identification of *sxt* genes in dinoflagellates became easier after the discovery of these genes in cyanobacteria (Hackett et al. 2013; Murray et al. 2015; Orr et al. 2013a; Stüken et al. 2011; Zhang et al. 2014). With the help of cyanobacterial *sxt* gene sequences, and use of transcriptomic annotation, PCR, and amplicon sequencing technologies, the identification and characterization of *sxt* homologs in dinoflagellate species became possible (Hackett et al. 2013; Murray et al. 2015; Orr et al. 2013b; Stüken et al. 2011; Zhang et al. 2014).

The first step of the PST biosynthesis pathway starts by the catalysis of the enzyme coded by the gene *sxtA,* one of the most studied genes in PST producing dinoflagellates (Murray et al. 2012; Stüken et al. 2011; Suikkanen et al. 2013; Wiese et al. 2014). *sxtA* contains four catalytic domains, encoded by *sxtA1* (SAM-dependent methyltransferase, MT), *sxtA2* (GCN 5-related

N-acetyltransferase, ACT), *sxtA3* (acyl carrier protein, ACP), and *sxtA4* (8-amino-7-oxononanoate synthase like class II aminotransferase, AONS) (Kellmann et al. 2008). Depending on the presence or absence of domain *sxtA4*, two isoforms of *sxtA* are found in dinoflagellates. One is the long transcript containing *sxtA1- A4* which is active in catalyzing STX biosynthesis (Murray et al. 2011) and other is the short transcript where *sxtA4* domain is absent (*sxtA1-sxtA3*) (Stüken et al. 2011). Three paralogs of *sxtA,* domain *sxtA1,* have been identified and among these three paralogs one has been reported to be directly linked with the PST production (Murray et al. 2015). It is believed that the presence of the domain *sxtA4* is the key to confirm a strain as PST producing strain as the absence of this domain has been confirmed in dinoflagellate genera or families which do not produce STX (John et al. 2014; Orr et al. 2013a; Stüken et al. 2011). The domain *sxtA4* was identified in the genome of non-toxic mutant of *A. pacificum*, though the expression of this gene was not observed in the mRNA during differential gene expression analysis (Zhang et al. 2014). No significant differential expression of the short transcript of *sxtA* was reported in the toxic and non-toxic *A. pacificum* (Zhang et al. 2014). *sxtG* was also identified as a vital gene in the PST biosynthesis pathway as the *sxtG* based phylogeny showed the presence of a highly conserved clade that comprise all the PST producing dinoflagellates (Murray et al. 2015).

Different factors like the increase of nitrogen concentration, phosphate limitation, turbulence, salinity stress, and presence of copepod can influence PST production by the PST producing *Alexandrium* species, though the clear reason has not been identified yet (Grzebyk et al. 2003; John & Flynn 2002; Juhl, Trainer & Latz 2001). It has been identified that in the presence of copepods, saxitoxin like bioactive compound production increase which indicates that these toxin compounds can provide a chemical defence in *Alexandrium* species to deter the grazers (Bagøien et al. 1996; Selander et al. 2006; Wohlrab, Iversen & John 2010; Yang et al. 2011). Copepods are also potential to produce chemical compounds called copepodamide that may act to give signal to the *Alexandrium* species to increase the production of PSTs (Selander et al. 2015).

Despite intensive studies performed to understand the regulation of the PST biosynthetic genes dinoflagellates, remains poorly understood. It has not been identified yet that whether the genes are regulated transcriptionally, post transcriptionally or translationally. To investigate *sxt* gene regulation, we have used a Nanostring gene array, in order to simultaneously measure the gene regulation in 18 individual probes representing the 8 'core' sxt analogs in Alexandrium pacificum and several housekeeping genes. Nanostring assays are based on a polymerase free nucleic acid quantification platform and hybridization technique which uses molecular barcodes and microscopic imaging to accurately detect and count the transcript abundance in a hybridization reaction(Geiss et al. 2008). We performed a comparative differential gene expression study between PST producing and non-producing *Alexandrium pacificum* strains in the presence and absence of cues from the copepod *Parvocalanus crassirostris*. The main purpose of this study was to do a detailed investigation of the expression of PST-related genes in *Alexandrium pacificum.*

3.3 Materials and Methods

3.3.1 *Alexandrium pacificum* strains

Four clonal strains of *A. pacificum* Litaker (*A. catenella* Group IV ribotype) were used in this experiment. Two of these strains were found to produce paralytic shellfish toxins (CS314/8, HRP2/17), while the other two did not produce paralytic shellfish toxins above the detection limit of LC-MS detection methods (CS316/3, CS798). Three strains were obtained from the Australian National Algae Culture Collection (CS strain numbers) and one was isolated in this study from Porto Bay, Hawkesbury River in 10.10.2017 as part of this project (HRP strain number) (Table 3.1). Details of culture isolation and establishment are given in Chapter 2. All strains were grown in K medium (Keller et al. 1987) without sodium silicate at 18°C under a photon flux of 60-100 µmol photon $m^{-2}s^{-1}$ on a 12/12 h dark/light cycle (white fluorescent tubes) prior to the experiment.

Table 3.1: Description of the strains used in the experiment

Strain	Site of culture isolation sample	Sample Collection Method	Toxicity Status
CS 314/8	Port Philip Bay, Victoria, Australia	Net haul sample	PST producer
HRP 2/17	Porto Bay, Hawkesbury River, NSW, Australia	Net haul sample	PST producer
CS 316/3	Ballast water, Kashima, Japan	Germinated from Cyst	No PST produced
CS 798	Port River, Adelaide, South Australia, Australia	Germinated from Cyst	No PST produced

3.3.2 Copepod strain

The calanoid copepod, *Parvocalanus crassirostris*, was obtained from the collection of the Australian Institute of Marine Science (AIMS). Pure copepod cultures were maintained in 1 μm filtered seawater (36 ± 1 ppt) with gentle aeration at 26 ± 1°C. Water salinity was maintained by adding dechlorinated distilled water as required.

3.3.3 Copepodamide identification

To check the compatibility of the copepods for the current experiment, the identification of the presence of copepodamide was performed using LC-MS and following the method used by Selander et al 2015.

3.3.4 Experimental design

The four *A. pacificum* strains (two toxic and two non-toxic), were grown in K media (without sodium silicate, (Keller et al. 1987) in 2 L conical flasks in triplicate. One set of flasks was designated the control treatment (total of 12 flasks), and the second, the experimental treatment (again 12 flasks). All twenty four flasks were kept at 18°C under a photon flux of 60-100 μmol photon $m^{-2}s^{-1}$ on a 12/12 h dark/light cycle (white-fluorescent tubes).

All replicate *A. pacificum* strains were grown until they reached a cell concentration of ~1500 cells/ml in all flasks. Due to the differing growth rates of each strain, this was achieved by a combination of media dilution and/or confirmation by cell count using a Sedgwick Rafter cell. Concurrent to this, batches of 20 adult copepods were isolated for each experimental treatment flask using light microscopy, and maintained in a petri dish with filtered seawater until further use. Once *A. pacificum* reached the target cell density of ~1500 cells/ml in all flasks, one batch of copepods was then introduced into each of the treatment flasks to induce toxin production (none were added to controls). To do this, each batch of copepods was introduced into polypropylene cages which had 40 μm mesh on both sides and held by a 40 cm string, and placed immediately into each of the treatment flasks. The cages were then raised and lowered gently inside the flask at least 5 times per day, ensuring that they did not rise above the level of the *A. pacificum* culture. Flasks were also slightly agitated several times per day to ensure equal light distribution. All flasks (control and treatment) were maintained for 48 hours before harvesting.

3.3.4 Harvesting

At the end of the experiment, the copepods were removed from the experiment flasks and their condition checked.

For RNA extraction, approximately 10^5 cells from all of the experimental flasks were harvested by slow centrifugation (at 2300 g for 10 min) during the exponential growth phase. Aggregated bacterial cells and sea water crystals were removed by washing the cell pellets with 1X PBS (Sigma, Australia), followed immediately by snap freezing in liquid nitrogen. The pellets were then stored in -80°C until the final RNA extraction.

For toxin analyses, approximately 500 mL of *Alexandrium* culture from each of the experimental flasks was centrifuged and washed with 1X PBS (Sigma, Australia). The resulting cell pellets were immediately frozen at −80 °C before freeze drying prior to transported to the Sydney Institute of Marine Science (SIMS), NSW, Australia, where 14 saxitoxin analogues were tested including STX (STX, C1, C2, GTX2, GTX1, dcGTX2, GTX3, GTX4, dcGTX3, GTX5, GTX6, dcNEO, dcSTX, NEO) by LCMS.

3.3.5 RNA extraction

During the RNA extraction, 1 ml of ice-cold TRIreagent (Life Technologies Australia Pty Ltd) was added to the cell pellet and heated at 60°C for 5 minutes. The frozen cells were lysed using bead beater homogenizer at top speed for 1 min. A further 200 µl of chloroform was added into the collected supernatant after bead beating. Centrifugation was performed for 10 min at 12000g at 4°C. The top aqueous layer was separated and mixed with equal volume of 100% isopropanol. This solution was incubated at -80°C for 2 hours before further processing. Purification steps were performed using RNeasy Mini Kit (Qiagen Pty Ltd) according to the supplier's guidelines. The purity and quantity of RNA were assessed using a Nanodrop (ND-1000, Thermo Scientific, Woltham, MA, USA). Extracted RNA was stored at -80°C for further gene expression analyses.

3.3.6 Nanostring nCounter, gene selection for toxin gene expression and gene expression analysis

Genes of interest associated with Paralytic Shellfish Toxins (PSTs) and reference genes with their associated sequences were selected based on the previously published papers (Supplementary document 4.1 for genes and sequences) and gene expression analysis was performed using the NanoString nCounter Expression analysis system (NanoString Technologies, USA) according to the manufacturer's instructions. The genes assayed were:

sxtA, sxtA1, sxtA4, sxtB, sxtD, sxtG, sxtH/T, sxtI, and *sxtU*. House keeping genes that were used in the assay were: CPH, DYN, ETIF, GTEF, ICDH, IPP.

Following a titration assay with RNA of three different samples from our experiment at four different concentrations (50 ng, 100 ng, 150ng, and 200 ng), the final PlexSet immunity assay was performed with 320ng of RNA as input using the purified RNA to their corresponding nCounter Reporter and Capture probes. A 16 h hybridization step was done with Veriti ABI PCR machine (Life Technologies Corporation, USA) at 65°C. The sample cartridge preparation was performed using the nCounter Prep Station (Nanostring Technologies). The individual fluroscent barcodes associated with the targeted genes were identified by an nCounter Digital Analyzer (nCounter® FLEX Analysis System, Nanostring Technologies). The machines were used according to manufacturer's instructions to capture direct digital counts of each gene in each sample. One sample ran in all PlexSets and was used to correct for PlexSet variation. GeNorm was used to assess the average expression stability of the targeted genes and the 6 most stable genes (*sxtA1-1, sxtU1-1, sxtl-1, sxtl-3*, GTEF and ICDH) were used in geomean to normalised gene counts. *sxtA1_1* is one of the paralog of *sxtA1* (Accession no. JF343242.1) that was reported to be present in both PST producing and non-PST producing species (Murray et al. 2015).

3.3.7 Toxin extraction and analyses

Toxin extractions were performed using the method of Harwood et al. (2013). Briefly, five millilitres of 1 mM acetic was then added to each sample tube and vortexed for 90 secs. The sample tubes were placed into boiling water bath for 10 min, and then cooled at room temperature. The samples were then placed in an ultrasonic bath for 1 min and finally centrifuged for 5 min. The supernatant was used for chromatographic separation on a Thermo Scientific™ ACCELA™ UPLC system (either with or without dilution).

A Thermo Scientific™ Q EXACTIVE™ high resolution mass-spectrometer equipped with an electrospray ionization source was used for the analysis. The following source parameters were used in all experiments: a capillary temperature of 263 °C, a spray voltage of 3.5 kV, an auxiliary gas heater temperature of 425 °C, a sheath gas and an auxiliary gas flow rate of 50 and 13 (arbitrary units). The mass spectrometer was operated in polarity switching mode scanning across the range of m/z 100–500. LC-MS was performed on a Thermo Scientific™ ACCELA™ UPLC system by the method published by Boundy et al. (2015)(Boundy et al.

2015). Separation was carried out by an Acquity UPLC BEH Amide 130 (150 mm × 2.1 mm i.d., 1.7 μm particle size) column. Mobile phases were A. Water: Formic acid: NH4OH (500:0.075:0.3) and B. Acetonitrile: Water: Formic Acid (700:300:0.1). The injected volume was 5 μL. Thermo Xcalibur software (version 3.0.63, Thermo Fisher Scientific, Inc.) was used for the data analysis. Analytical standards for the PST analogues were obtained from National Research Council, Canada.

3.4 Results

3.4.1. Confirmation of the presence of Copepodamide

The copepod *Parvocalanus crassirostris* was confirmed to produce copepodamide using LC-MS. Five different copepodamide compound were identified in *P. crassirostris* which include copepodamide A (molecular wt. 761.5), copepodamide B (Molecular wt. 735.5), copepodamide C (Molecular wt 709.5), copepodamide D (Molecular wt.759.5), copepodamide E (Molecular wt. 735.5).

3.4.2. Cellular PST profiles in *Alexandrium pacificum*

The *A. pacificum* strains CS314 and HRP24 were confirmed to produce PSTs using LC-MS. Cell specific toxin content showed the presence of the PST analogues, C1, C2, GTX1, dcGTX2, GTX3, GTX4, GTX5, and GTX6 in different concentrations in both strains (Table 3.2). The same PST analoges were identified in both the control and treatment conditions, with a high concentration of C1 analogue content. Strain CS314 produced a greater amount per cell compared to HRP24 (Table 3.2). In the treatment condition, two analogues C2 and GTX4 were identified to be present in high concentration.

Strains CS798 and CS316 did not produce any known PST analogs in either the control or treatment condition, at a detection limit of 0.01 pg/cell per cell (Table 3.2).

Table 3.2: PST amounts per cell of each toxin analog of two strains of *Alexandrium pacificum.* Toxins are in pg/cell. The limit of detection was 0.01 pg/cell. PSTs in the strains CS798 and CS316 were below the limit of detection.

Strain	Condition	C1	C2	GTX1	dcGTX2	GTX3	GTX4	GTX5	GTX6	Total Toxin
CS314A	Control	5.1	22.4	2.0	0.2	0.3	9.5	1.7	4.3	45.6
CS314B	Control	1.2	17.3	2.4	0.2	0.3	11.0	1.3	3.3	37.1
CS314C	Control	0.9	24.5	2.0	0.0	0.7	13.0	1.7	4.2	47.0
CS314D	Treatment	1.7	52.1	5.3	0.0	1.7	32.5	4.1	10.3	107.7
CS314E	Treatment	4.4	60.7	5.6	0.2	0.0	24.0	0.0	6.7	101.5

CS314F	Treatment	1.2	38.4	2.4	0.0	1.2	20.5	2.7	8.2	74.6
HRP24A	Control	0.8	33.8	0.3	0.1	0.0	2.5	0.1	0.5	38.1
HRP24B	Control	0.4	14.6	0.1	0.1	0.1	1.0	0.2	0.2	16.7
HRP24C	Control	0.3	3.6	0.0	0.1	0.0	0.3	0.1	0.0	4.5
HRP24D	Treatment	1.6	69.9	0.4	0.1	0.2	4.8	0.1	0.0	77.0
HRP24E	Treatment	1.5	51.6	0.5	0.1	0.0	5.7	0.1	1.0	60.5
HRP24F	Treatment	0.4	7.3	0.1	0.1	0.1	0.5	0.2	0.1	8.8

For both strains, the amount of toxicity was greater in the treatment condition than in control After 24 hours exposure of *A. pacificum* strains CS314 and HRP24 to the copepods, cell specific PST content significantly increased (Two-way ANOVA $p < 0.05$, Tukey's post-hoc $p < 0.05$, Two-way PERMANOVA $p < 0.05$).

In strain HRP24, the concentration of the analogues C1, C2, GTX1, GTX4, and GTX3 were significantly higher (Two-way ANOVA, $p < 0.05$) compared to the treatment. In the strain CS314, the concentration of the analogues C1, GTX1, GTX4, GTX6 were significantly higher compared to the treatment (Two-way ANOVA, $p < 0.05$). Conversely, in a copepod PST-induction experiment conducted on the species *A. minutum*, GTX4 and GTX1 was reported as the dominating PST derivatives and the composition of PST analogues was identified as the same in the control and treatment (Selander et al., 2006).

No significant changes were observed for CS798 and CS316 after the grazing experiment.

3.4.3 Gene expression profile

Notable gene expression variation was observed for 3 genes *sxtA, sxtA1_2,* and *sxtA4* between the toxic (CS314 and HRP24) and non-toxic (CS798 and CS316) strains (Figure 3.1). These 3 genes were identified as upregulated in CS314 and HRP24 regardless of the experimental condition (Figure 3.1). However, for the highly toxic strain CS314, these 3 genes were identified to be expressed more than the other toxic strain HPR24 (Figure 3.1). A significant downregulation trend was also observed in the non-toxic CS798 and CS316 strains for these same three genes despite the presence or absence of the copepods. No other genes showed any significant trend between the control and treatment conditions; however, most genes were downregulated in the non-toxic strains compare to the toxic strain (Figure 3.1). In the toxic strain CS314, the expression of the genes *sxtB_2, sxtG1, sxtH.T_4,* and *sxtI_2* was higher than HRP24 in both experimental conditions. Again, in HRP24, *sxtB_1, sxtH.T_3, sxtI_3,* and *sxtU_1* genes expressed more than CS314 (Figure 3.1).

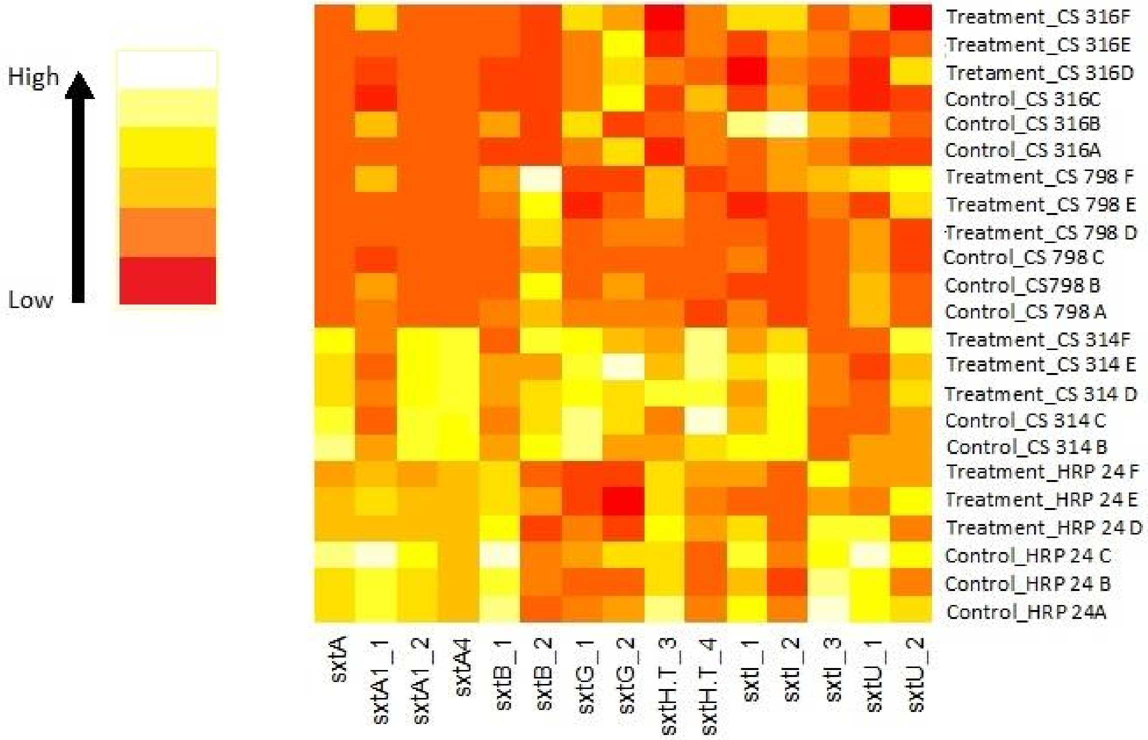


Figure 3.1: Gene expression profile of 4 *Alexandrium pacificum* strains in control and copepod exposure conditions.

A principal coordinate analysis (PCA) demonstrated clear separated clusters between the expression level of genes between the toxic and non-toxic *A. pacificum* groups irrespective of the control and treatment conditions, with 95% circle ellipse (PC1 = 91.21%, PC2 = 5.99%, Figure 3.2A). Moreover, a significant separation was observed among all the strains, although no difference was observed between control and treatment conditions (95% circle ellipse, PC1 = 91.21%, PC2 = 5.99%, Figure 3.2B).

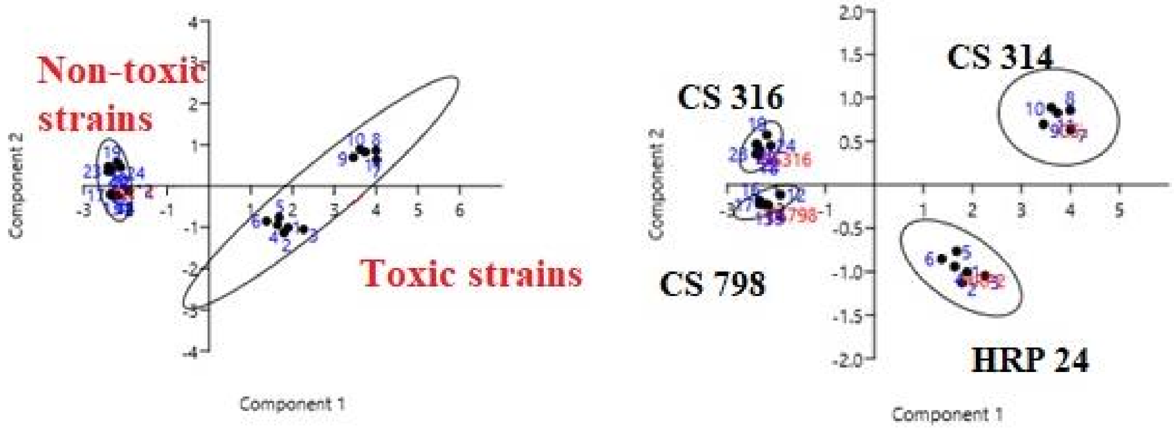


Figure 3.2. Results of PCA using normalized data. **A.** PCA of toxic (CS314, HRP24) and non-toxic (CS798 and CS316) strains with 95% circle ellipse. **B.** PCA of different strains of *A. pacificum* with 95% circle ellipse. Clustering separation was not observed for the control and treatment conditions.

A significant change in the toxin production related gene expression was observed between the toxic and non-toxic strains (Two-way PERMANOVA, $p = 0.0001$). However, the presence of copepod cues did not influence the gene expression variation, hence, no significant difference of gene expression was observed between control and treatment.

Similarity of percentage (SIMPER) analyses of the normalized data between toxic and non-toxic *A. pacificum* strains showed that genes *sxtA_2* (39.5%) and *sxtA4* (16.4%) contributed the most to the difference between these two groups (supplementary table 4.2)

3.4.4 Correlation between toxin content and gene expression

The expression of several genes was identified to be positively correlated with toxin content. *sxtA4* was identified as mostly correlated with toxin content (Figure 3.3). The expression of *sxtA, sxtA1_2,* and *sxtH.T_4* also showed a good positive correlation with the amount of toxin. No negative correlation between the toxin content and gene expression was found during this correlation test.

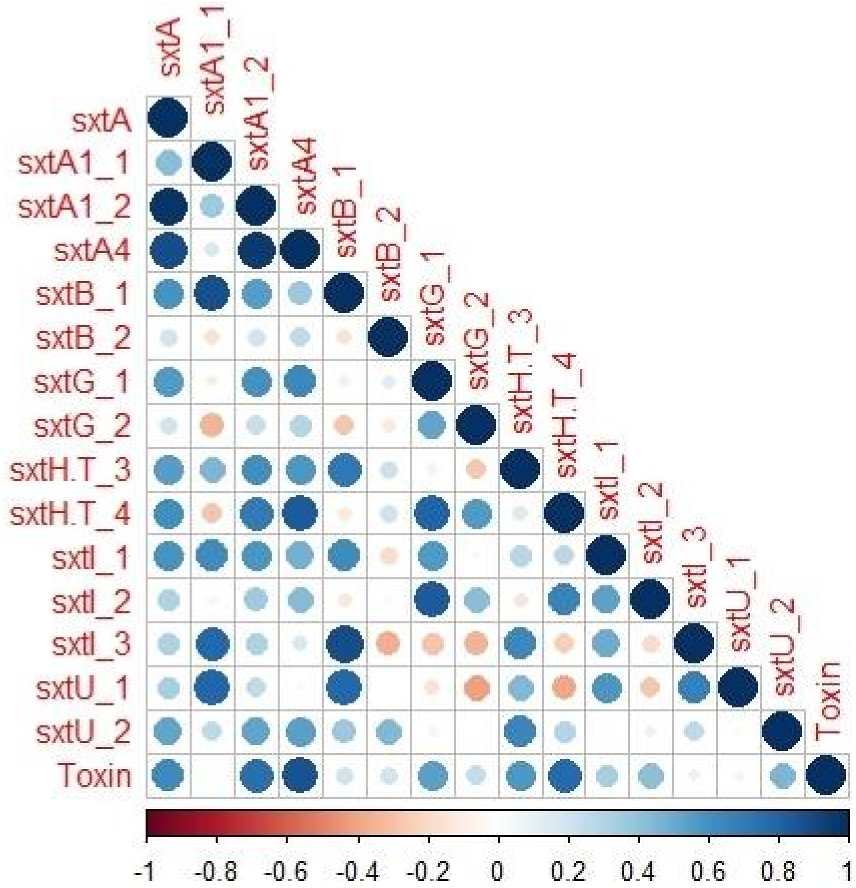


Figure 3.3: Correlation between the genes related to toxin production and toxin content.

3.5 Discussion

In this study, we examined the expression of multiple potential PST-related genes in relation to PST production using a novel NanoString assay, by exposing strains of *Alexandrium pacificum*, comprising PST producing and non-producing strains, to a treatment involving copepod cues, a known inducer of increased PST production. The exposure of strains of *Alexandrium pacificum* to *Parvocalanus crassirostris* led to a significant increase the amount of PSTs produced by PST producing strains of *Alexandrium pacificum,* though no significant increase in transcript abundance were observed in these strains. No change in PST production or transcript abundance were observed in the previously non-PST producing *A.pacificum* strains. A significantly higher transcription of *sxt* genes was found in the PST producing *A.pacificum* strains, compared to the non-producing strains.

Copepod induced toxicity in *Alexandrium pacificum*

This study clearly demonstrated that this population of the calanoid copepod *Parvocalanus crassirostris*, which commonly occurs in Australian coastal water and can significantly increase the cell specific PST production of strains of *Alexandrium pacificum*. The chemosensory recognition of the *A. pacificum* strains (CS314 and HRP24), isolated from Port Philip Bay, Victoria and Hawkesbury River, New South Wales, Australia, respectively against this prey copepod resulted in the formation of about 2.2 times higher PST concentrations compared to the controls in a 48 hr incubation experiment, which was significantly greater compared to controls (Significant test was performed by a Two Way PERMANOVA test) Table 3.2. In previous studies, other calanoid copepods *Acartia tonsa, Acartia clausii, Centropages typicus, Calanus helgolandicus* were successfully used to induce PST toxicity in different *Alexandrium* species, and about 2 to 20 times more PST production was observed in treatment compared to the control, which was found to statistically significant in those studies (Bergkvist, Selander & Pavia 2008; Selander et al. 2006; Wohlrab, Iversen & John 2010; Wohlrab, Selander & John 2017; Yang et al. 2011). To our knowledge, *Parvocalanus crassirostris,* a member of paracalanoide family, has been used in the current study for the first time for PST induction in *Alexandrium pacificum.* This copepod was identified to produce five different copepodamide compound (A-E) which can give the chemosensory stimulation to *Alexandrium* cells (Selander et al. 2015). *Alexandrium* cells can show a chemical defence against the copepods by increasing the saxitoxin synthesis (Bagøien et al. 1996; Selander et al.

2006; Wohlrab, Iversen & John 2010). It has been reported that the presence of pico to nano molar concentration of copepodamides can give up to 20 fold increase of PSTs (Selander et al. 2015).

The PST analogue C2 was identified as the dominant analogue in terms of quantity followed by GTX4 and GTX6, in both CS314 and HPR24 strains, which were isolated from Port Philip Bay, Victoria and Hawkesbury River, New South Wales, Australia, respectively (Table 3.2). This is a similar PST profile to other strains of *Alexandrium pacificum* from the Pacific region (Barua et al 2020, Murray et al 2015, Bolch et al 2018). The strains CS314 and CS798 are two rare non-toxic strains, in that they were not found to produce any detectable PSTs. In the literature, this is very uncommonly reported for strains of *Alexandrium pacificum* (Parker 2002, Ruvindy 2019) , having only ever been reported in strains isolated from Japan and China (Tsuchiya et al 2014, Omura et al 2003, Zhang et al 2014). In each case, these strains were originally germinated from cysts, rather than swimming cells, in one case as a result of a laboratory crossing experiment among strains (Parker 2002, Omura et al 2003).

The experimental copepod-exposure PST induction treatment increased the concentration of different PST analogues at different rates, without following any significant pattern. In strain HRP24, the concentration of the analogues C1, C2, GTX1, GTX4, and GTX3 are significant (Two-way ANOVA, $p < 0.05$) compare to the treatment. In the strain CS314, the concentration of the analogues C1, GTX1, GTX4, GTX6 are significant compare to the treatment (Two-way ANOVA, $p< 0.05$. Conversely, in a copepod PST-induction experiment conducted on the species *A. minutum,* GTX4 and GTX1 was reported as the dominating PST derivatives and the composition of PST analogues was identified as the same in the control and treatment (Selander et al., 2006).

However, the non-PST producing *A. pacificum* strains CS798 and CS316 did not produce any detectable PST analogues following exposure to the copepod treatments. This result suggests that these strains lack genetic mechanisms to regulate PST production as a defensive action in the presence of copepods. These two strains both possess genes encoding *sxtA*, including the domain sxtA4 (Ruvindy 2019)(Ruvindy et al, 2019).

These findings suggest that copepods can induce additional PST production in *Alexandrium pacificum* only when the strains are already capable of PST production, as they have both the relevant genes (*sxt*) and the appropriate regulatory mechanisms. The increase in PST concentration by chemosensory stimulation of the PST producing *Alexandrium sp* could allow for an opportunistic environment for all the genetically related *A. pacificum* cells in an entire bloom irrespective of less toxic and/or nontoxic strains (Selander et al. 2006; Wolfe et al. 2000).

PST related gene expression profile in *Alexandrium pacificum*

In the present study, the expression of 18 PST related core genes was examined, including more than one paralogue for one specific gene. The first gene that initiates the PST biosynthesis pathway in dinoflagellates is *sxtA*, which has been identified as the pivotal gene in toxin biosynthesis in several studies (Cho et al. 2019; Cirés et al. 2017; Murray et al. 2015; Stüken et al. 2011; Tsuchiya et al. 2017; Zhang et al. 2014). In our current study, the involvement of *sxtA* is demonstrated again. We have selected four *sxtA* related genes: the long isoform of *sxtA*, which includes the domain sxtA4, (Accession no KM100455.1)*,* two short isoforms of *sxtA*: s*xtA1_1* (Accession no JF343242.1), and *sxtA1_2* (Accession no JF343240.1), and the domain *sxtA4* (KF164508.1) on its own. A significant variation of transcript count and variation in gene expression among *A. pacificum* strains was observed in this study (Figure 3.1).

The long isoform of *sxtA, the* short isoform of *sxtA* (*sxtA1_2* in our study), and *sxtA4* are present in both PST producing and non-PST producing *A. pacificum* were all expressed (Figure 3.1). However, the PST producing strains showed a significantly higher abundance of transcipts compared to the non-PST producing strains (Figure 3.1, & 3.2 , P <0.001). The transcript abundance was positively correlated with the gene copy number (Bachvaroff & Place 2008) which means the PST producer *A. pacificum* stains have a high number of the long isoform of *sxtA,* short isoform of *sxtA* (*sxtA1_2* in our study), and *sxtA4.* Similarly, we can confirm that the non-PST producer *A. pacificum* strains also have these particular genes with a low transcript number.

The results differ with previous studies where it has been identified that absence of part, or all of *sxtA* in the *sxt* gene cluster, is responsible for the inability to produce STX in dinoflagellate genera and families (John et al. 2014; Murray et al. 2015; Orr et al. 2011; Stüken et al. 2011). A study on *Alexandrium ostenfeldii* showed that the strains which do not have the domain *sxtA4* cannot produce STX (Suikkanen et al. 2013). However, in these strains, the lack of PST production and the inability to induce PST production in these particular strains appears to be due to gene regulatory factors, rather than the lack of s*xtA4*.Another study revealed that a high expression of the long isoform of *sxtA* is directly involved in PST biosynthesis, whereas the down regulation of the long isoform of *sxtA* is responsible for the inability of PST biosynthesis in the nontoxic *A. pacificum* (Zhang et al. 2014). The current study has confirmed this hypothesis. Wohlrab et al 2017 stated that in the STX biosynthesis pathway, *sxtA* may not be a key gene. However, in our study, based on SIMPER test we have identified that, the genes *sxtA1_2 and sxtA4* were the highest contributor compare to the other included genes and the values are 39.5% and 16.4 % respectively. The results also revealed that *sxtA1_2* and *sxtA4* have a higher gene count in more toxic cs314 strain compared to HRP24.

sxtG is considered another important gene in the *sxt* gene cluster. *sxtG* was thought to be absent or a poorly transcribed gene in the non-PST producing dinoflagellates genera (Orr et al. 2013b). Murray et al (2015) identified only one highly conserved clade in the *sxtG* phylogeny which comprised all of the PST producing dinoflagellates. In the current study, we have established the expression of *sxtG* in all *A. pacificum* strains, irrespective of PST production levels (Figure 3.1) (Murray et al. 2015). We have identified that for gene *sxtG_1* (Accession no JV310276.1), the transcript abundance is higher in the comparatively more toxic CS314 strain than in HRP24 (Figure 3.1). No significant difference was observed between HRP24 and both non-PST producing strains.

Addditionally, we did not identify any difference in expression of the potential PST production related genes *sxtB_1, sxtB_2, sxtG_2, sxtH/T_3, sxtI_1, sxtI_3, sxtU_1,* and *sxtU_2* in relation to the strains or the treatment conditions (Figure 3.1, 3.2). Two other genes, *sxtH/T_4* and *sxtI_2*, showed significantly higher expression in CS314 than other strains. The enzyme produced from the gene *sxtH/T* is reported to form the first STX derivative in the biosynthesis pathway named as dcSTX and *sxtI* is identified to form the enxyme that helps to convert dcSTX to saxitoxin (Kellmann et al. 2008). Therefore, it is possible that genes of the *sxt* gene cluster might be expressed more in the highly toxic strains compare to the low toxic or nontoxic strains.

In a study of *A. minutum* isolated from the Mediterranean area, no correlation was observed between *sxtA4* and the toxin content at the transcriptional level (Perini et al. 2014). Conversely, we identified a positive correlation between the amount of PST produced and the expression of almost all of the genes used in this study (Based on pearson correlation Figure). Among them, the correlation with the genes *sxtA4, sxtA1_2, sxtA, sxtH/T_4,* and *sxtG_1* was higher than the correlation with other genes (Based on perarson correlation, Figure 3.3)

In the present study, exposure of strains of *Alexandrium pacificum* to the calanoid copepod *Parvocalanus crassirostris* led to a significant increase the amount of PSTs produced by strains of *Alexandrium pacificum,* though no significant changes in gene expression was observed at the transcriptomic level. Similarly, no gene expression change was found in the non-PST producing strains. This suggests that PST production might not be regulated at the transcriptional level in strains of *Alexandrium pacificum*, but rather at a post-transcriptional level. In previous studies, it has been suggested that a minority of dinoflagellate genes, ~5 to 30 % of genes are regulated at the transcriptional level (Johnson et al. 2012; Lin et al. 2010). In dinoflagellates, translational regulation has been reported for several physiological processes which includes bioluminescence, carbon fixation, photosynthesis, and the cell cycle (Le et al. 2001; Nakamura et al. 1989; Van Dolah et al. 2007). In a recent iTRAQ based quantitative proteomic study has suggested that PST biosynthetic pathway may not be regulated translationally, and chances are high that it might be regulated post-translationally (Zhang et al. 2018). The possibility of post-translational regulation is strong because of the presence of trans-splicing process where a specific 22 bp oligonucleotide spliced leader can be added to the 5’ end of a heterogenous RNA group (Morey et al. 2011; Zhang, Bhattacharya & Lin 2007).

Chapter 4:

Population genetic structure of *Alexandrium pacificum* in two different Australian boundaries current

4.1 Abstract

Certain species of *Alexandrium* are widely known due to their production of paralytic shellfish toxins (PSTs). The appearance of *Alexandrium pacificum* is now frequent in coastal waters of Australia, particularly in New South Wales and Western Australia. An understanding of the population dynamics of species of *Alexandrium* can unveil evolutionary, demographic, and adaptive processes in these economically important dinoflagellates. Using Single Nucleotide Polymorphisms (SNPs) identified from transcriptomes of the strains, this study identified distinct, genetically isolated populations of *Alexandrium pacificum* associated with sites from the major southward-flowing Australian boundary currents, the East Australian Current, (EAC), and the Leeuwin current (LC) on the western and southern coastlines. We identified the presence of distinct clusters formed by the strains isolated from EAC sites and LC sites. This genetic isolation indicates a relationship between the formation of cyst beds of local populations which may initiate HAB formation under favourable environmental conditions. However, there was no significant relationship between the clusters of *A. pacificum* strains and differing capacities for PST production or PST profiles. Future research using a greater number of isolates and the PST (*sxt*) genes may help to further elucidate the differentiation and gene flow among populations and the methods with which population differentiation is maintained.

4.2 Introduction

Marine phytoplankton communities are generally recognised to be ubiquitous across open pelagic habitats as ‘free drifters’. Their large population sizes along with high dispersal capacity can be facilitated by different oceanic current systems, the absence of any visible dispersal barriers, and having a ‘bloom-and-bust’ life history strategy (Cermeño & Falkowski 2009; Finlay 2002; Norris 2000; Smayda & Reynolds 2003). Such enormous distribution ability has raised the argument that marine eukaryotic microbes may have little, if any genetic partitioning due to high gene flow, and hence may be the same all over the globe; which conflicts with the idea of local adaptation and allopatric speciation (Palumbi 1992; Whittaker & Rynearson 2017). However, with the advancement of genomics and transcriptomics, these arguments have been partially resolved, as it has been revealed that certain marine eukaryotic microbial species can demonstrate high genetic diversity with substantial trait variation both across spatial and temporal gradients (Alpermann et al. 2010; Gao et al. 2019; Nagai et al. 2007; Paredes et al. 2019).

Among diversified marine microbial eukaryotes (protists), dinoflagellates are widely known for their ability to produce toxins as part of their harmful algal bloom (HAB) formation (Hallegraeff 1993; Smayda 1997a; Smayda 1997b). It has been reported that about 2% of algal species are responsible for the HAB events worldwide, and that ~75% are comprised of dinoflagellates (Heisler et al. 2008; Smayda & Reynolds 2003). Some species of the genus *Alexandrium* Halim has been identified as the most common HAB forming dinoflagellates (Anderson, Cembella & Hallegraeff 2012; Farrell et al. 2013). They can produce a variety of bioactive substances known as paralytic shellfish toxins (PSTs) which can cause Paralytic Shellfish Poisoning (PSP) (Kao 1993). PSP is considered to be a global seafood safety issue for its negative impact on human health and aquaculture industries (Anderson, Cembella & Hallegraeff 2012). Besides PSP, some *Alexandrium* species show negative effects on unicellular eukaryotic organisms by producing extracellular allelochemicals (Ma et al. 2009; Tillmann & John 2002a). Evidence shows that over past 30 years, the frequency, geographic range and intensity of HAB events have been significantly increased (Hallegraeff 1993; Hallegraeff et al. 2010; Smayda 1990). Moreover, some species of *Alexandrium* are likely to persist in many regions despite changing environmental conditions (particularly higher water temperatures), with an indication of increased toxicity (Kremp et al. 2016; Masseret et al.

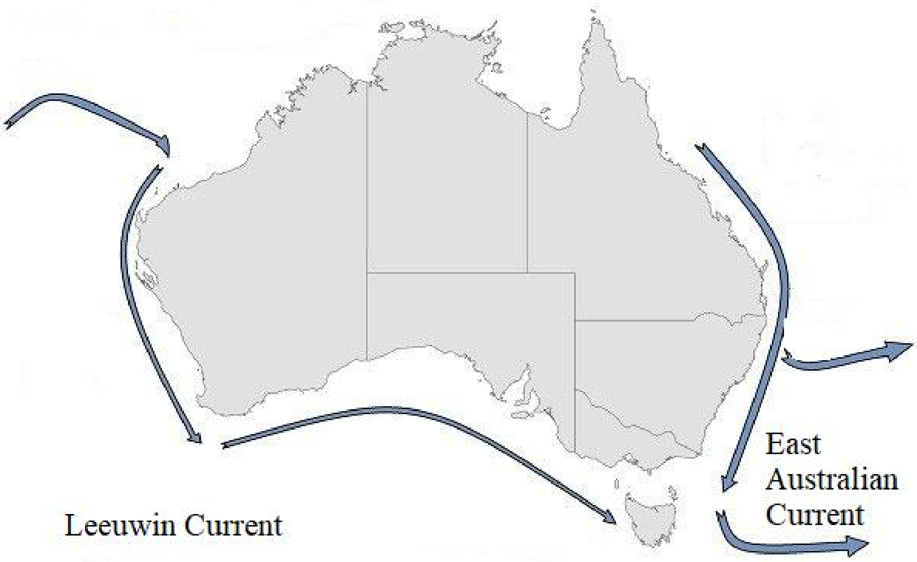
2009). For this reason, there is an imperative to understand the importance of the diversity of toxin production and genetic variability in PST producing species of *Alexandrium,* in order to understand the consequences of this on future HAB formation in a region.

Population genetics studies of some marine HAB have shown that phytoplankton are both genetically and phenotypically diversified, and that this diversity is highly structured on both spatial and temporal scales (Alpermann et al. 2010; Casabianca et al. 2012; Gao et al. 2019; Nagai et al. 2007; Richlen et al. 2012). Population genetic studies have been conducted on several species of *Alexandrium* using microsatellite markers or AFLPs to investigate population diversity (Casabianca et al. 2012; Nagai et al. 2007; Paredes et al. 2019). It was observed that even ecologically relevant traits from a particular geographic population exhibited high levels of genotypic and phenotypic variation with a significant multilocus linkage disequilibrium (LD) (Alpermann et al. 2009; Alpermann et al. 2010; Richlen et al. 2012).

In terms of spatial differentiation, the co-occurrence of multiple genetically distinct clusters confirmed the limited migration potential of the vegetative cells of *Alexandrium catenella* in the Mediterranean Sea and *A. minutum* in the Gulf of Maine of northwest Atlantic (Casabianca et al. 2012; Gao et al. 2019). The presence of oceanographic barriers was indicated as the possible reason for the genetic isolation among populations, as this could eventually restrict the gene flow via natural dispersal (Casabianca et al. 2012). Current systems have been shown to allow for gene flow along a gradient, showing patterns such as isolation by distance in studies of *A. catenella* (Paredes et al. 2019). Such diversity gradients have also been seen in dinoflagellate cysts, that is, the resting stages of dinoflagellates, that have preserved high genetic diversity with relatively restricted gene flow, a phenomenon that has been called "the anchoring effect" (Sjöqvist et al. 2015). In response to adverse environmental conditions, *Alexandrium* reproduces sexually and forms these dormant resting cysts (Anderson, Cembella & Hallegraeff 2012). The recombination of genetic material may happen within geographically closely related individuals during this sexual stage of life cycle, which allows the supply of the strains with novel genotypes as soon as the excystment is initiated (Anderson, Cembella & Hallegraeff 2012; Kremp et al. 2016). Sexual reproduction of this type has also been shown to greatly impact the demography and population genetic structure in the species *A. minutum* in two different estuaries of France (Dia et al. 2014).

In addition to diversity at the spatial scale, temporal differentiation has also been observed in dinoflagellate populations. Higher inter-annual genetic differentiation was found in *A. minutum* than the diversity during bloom events, although the latter diversity was also higher than expected (Dia et al. 2014). Temporal genetic differentiation was also found in blooms of *A. catenella* on shorter time scales (Erdner et al. 2011; Richlen et al. 2012). While the causes of this temporal diversity may relate to advection due to currents, spatio-temporal variation of *A. catenella* population structure might also occur through selection on genotypes related to environmental conditions like changes in the concentration of nutirients such as nitrite, nitrate, and silicate (Gao et al. 2019). Significant variability has been observed coinciding with an increase in temperature and a reduction/raise of salinity during the bloom season in different species of *Alexandrium* possibly indicating local adaptation (Gao et al. 2019; Kremp et al. 2016; Masseret et al. 2009). The evolution of phenotypic traits such as morphological characters, PSTs or allelochemicals production might be linked to the genetic diversity of *Alexandrium* populations (Alpermann et al. 2009; Alpermann et al. 2010; John et al. 2014; Le Gac et al. 2016). Substantial intraspecific variation in toxicity was reported in genetically different strains of *Ostreopsis* species, a palytoxin-producing dinoflagellate related to *Alexandrium* (Verma et al. 2020). Like *Alexandrium*, populations of *Gambierdiscus caribaeus* showed high temporal genetic variation which was linked with salinity changes in the benthic habitat (Sassenhagen et al. 2018). This latter study discovered more temporal differentiation than spatial differentiation in *G. caribaeus* populations (Sassenhagen et al. 2018). Apart from factors such as transport via ocean currents and local environmental pressures, grazing pressure and parasitism have been reported to contribute to temporal changes in *Alexandrium* population structure (Richlen et al. 2012). To survive under changing environmental conditions, successful adaptation of marine organisms has been recorded which might be responsible for the differentiation of population structure (Frusher et al. 2014).

In 2012 Australia experienced its most significant HAB event caused by *Alexandrium catenella*, which resulted in PST uptake in mussels, oysters, abalone, crabs and lobster, at levels of upto 10 mg/Kg, which cost the Australian industry AUD ~$23 M in lost revenue (Bolch et al. 2014; Campbell et al. 2013). In 2016, another significant HAB event was reported. Species of *Alexandrium* was detected in New South Wales (NSW), south eastern Australia, which persisted for at least eight weeks (Barua et al. 2020). This time the causative dinoflagellate was *A. pacificum* and the highest PST level in mussels was found to be 7.2 mg/kg (Barua et al. 2020). Both events occurred in the EAC region, which has been found to be home to multiple PST producing *Alexandrium* species, including these two, as well as *A. australiense, A.ostenfeldii* and *A.minutum* (Farrell et al. 2013).



4.1 Two Australian Boundary currents: East Australian Current (EAC) and Leeuwin Current (LC)

Australia is surrounded by two major boundary currents, namely the Eastern Australia Current (EAC) and Leeuwin Current (LC), between 27.5° and 34°S latitude, and travelling along the eastern and western coast respectively (Condie, Oliver & Hallegraeff 2019; Thompson et al. 2011) (Figure 4.1). Over the past 50 or so years, the EAC has become known as a ‘hot spot’ for ocean climate change (Ridgway & Hill 2012; Ridgway 2007). As a consequence, marine ecosystems have been significantly affected (Suthers et al. 2011). The EAC has stronger upwelling and a higher nutrient concentration compared to the LC, which controls phytoplankton productivity and distribution, and is directly connected with coastal phytoplankton blooms in the EAC region (Hallegraeff & Reid 1986; Koslow et al. 2008; Suthers et al. 2011; Thompson et al. 2011). The EAC favours spring phytoplankton blooms across the east coast during changing seasonal conditions, whereas the south-eastern region of Australia does not experience any spring bloom due to the involvement of LC (Thompson et al. 2011). The differences in the physical and chemical parameters of these two current systems may, therefore, impact the selection pressures on *Alexandrium* species present in these two major boundary currents. In addition, these current systems may act as a physical barrier, inhibiting the transport of cells. It has been identified that these two boundary currents have a strong influence on the genetic structuring of sea urchin along the Australian coastline (Banks et al. 2007). Surface temperature variability and geography have also been shown to influence the connectivity of the sea urchin population (Coleman, Roughan, et al. 2011). Therefore, understanding the influence of the EAC and/or LC on the connectivity of *Alexandrium* populations is important and currently a knowledge gap in our understanding of HAB species distribution, genetic diversity and bloom potential.

Different genetic markers have been used to estimate the genetic diversity within and the differentiation among populations of different *Alexandrium* species. In some studies, AFLP (Amplified fragment length polymorphisms) have been used for genetic characterization of the populations of *A. catenella* (sometimes as *A tamarense*) (Alpermann et al. 2009; Alpermann et al. 2010; Paredes et al. 2019). In other studies, rRNA gene markers (ITS1, 5.8S and ITS2. D1/D3 28S) have been used to identify genetic diversity and to determine population structure (Dia et al. 2014; Masseret et al. 2009). Microsatellites have been considered as the most widely used markers to assess the spatial and temporal scale genetic diversity of *A. catenella*, *A. tamarense*, and *A. minutum* across different studies (Alpermann et al. 2010; Casabianca et al. 2012; Erdner et al. 2011; Gao et al. 2019; Masseret et al. 2009; Nagai et al. 2007; Richlen et al. 2012). Single-nucleotide polymorphisms (SNPs) has emerged more recently as an informative genetic marker for population genetic studies (Liu et al. 2005). The robustness and better presumption quality has made SNP markers popular to identify the genetic variation of the organisms with complex traits (Fischer et al. 2017; Liu et al. 2005). Dinoflagellates possess a complex genomic structure, with amongst the largest known genomes (1.5 to 185 Gbp), a large number of chromosomes (4 to 220), and containing a high gene copy number (Casabianca et al. 2017; Verma et al. 2019). Due to these unique features, it is difficult to conduct complete genome sequencing of dinoflagellates (Verma et al. 2019). Therefore, for genomic level studies or gene identification in dinoflagellates, transcriptomic studies have proven to be a feasible approach (Murray et al. 2012).

In this present study, we characterized the *Alexandrium pacificum* population structure and connectivity across sites from both the EAC and the LC, the east and west coasts of Australia. To assess the genetic diversity, we performed RNA-seq analysis of 28 strains of *A. pacificum* isolated from 5 different locations. Based on RNA-seq results, we have identified the distribution of SNPs on the transcribed regions of the several strains. Using the SNP dataset, we determined the phylogenetic relationships among the strains isolated from Australian coasts and one from cyst germinated strain. Furthermore, we have identified the genetic diversity associated with differences in PST production and other important phenotypic traits. This study will cast further light to understand the evolutionary changes during climate change and the influence of genetic variation to form phenotypically diverged populations.

4.3 Materials and Methods

4.3.1 Sample collection, *Alexandrium* cell isolation and culture establishment

In this study, 28 strains of *Alexandrium pacificum* were used to investigate the population structure of this species in Australia. Among them, 26 non-axenic monoclonal cultures were established from vertical phytoplankton net haul samples (20 µm mesh) collected during various *Alexandrium pacificum* blooms in Australia that occurred during the course of this study (Table 4.1, Figure 4.2). If a suspected HAB of an *Alexandrium* species was noticed by a local council, shellfish safety program or water monitoring body, samples were collected by collaborators on site, and immediately shipped to UTS for species identification and strain isolation. The single *Alexandrium* cells were isolated using a Nikon Eclipse TS100 inverted microscope (10x magnification) by a drawn-out glass pipettes (pasteur pipettes) (Anderson & Kawachi 2005) and transferred into Falcon® 24 well culture plates containing 1 ml of 5x diluted K medium (Keller et al. 1987) without sodium silicate, to facilitate growth. Additionally, germanium dioxide was added at a concentration of 5 µg/ml to establish diatom free cultures. The multi-well culture plates were placed at 18˚C under cool white fluorescent light with a photon flux of 60-100 µmol photon m^{-2} s^{-1} on a 12/12 hour dark/light cycle. The growth status of the isolates was checked every alternate day. Once the cell density of the isolates was high enough, the cultures were transferred into 20 ml of K medium in a 70 mL gamma sterile polystyrene container with polyethylene caps (Thermo Fisher Scientific, Australia, Pty), and allowed to grow in the same temperature and light conditions outlined above. To maintain healthy growing cultures for further experiments, subculturing of the strains were continued into K medium every three weeks.

Table 4.1 Details of bloom location, collection date, number of strains established and strain identification. Note: NSW=New South Wales; WA=Western Australia; SA=South Australia.

Location of bloom	Coordinates of the location (lat, long)	Date of collection	Isolated from	No of established cultures	Strain ID
Porto Bay, Hawkesbury River, NSW	-33.564871, 151.220101	10.10.2017	Vegetative cell	3	HRP
Twofold Bay, NSW	-37.086928, 149.910880	02.08.2018	Vegetative cell	8	TFB
Mindarie Marina, WA	-31.690558, 115.701580	22.11.2018	Vegetative cell	15	MMWA

Sydney Harbour, NSW	-33.855887, 151.252680	Culture collection, collected in January 1993 by S.Norwood	Vegetative cell	1	ACSH02
Port River, Adelaide, SA	-34.762061, 138.511998	ANACC Culture Collection, collected in July 1997, by N. Parker	Cyst from sediment sample	1	CS798

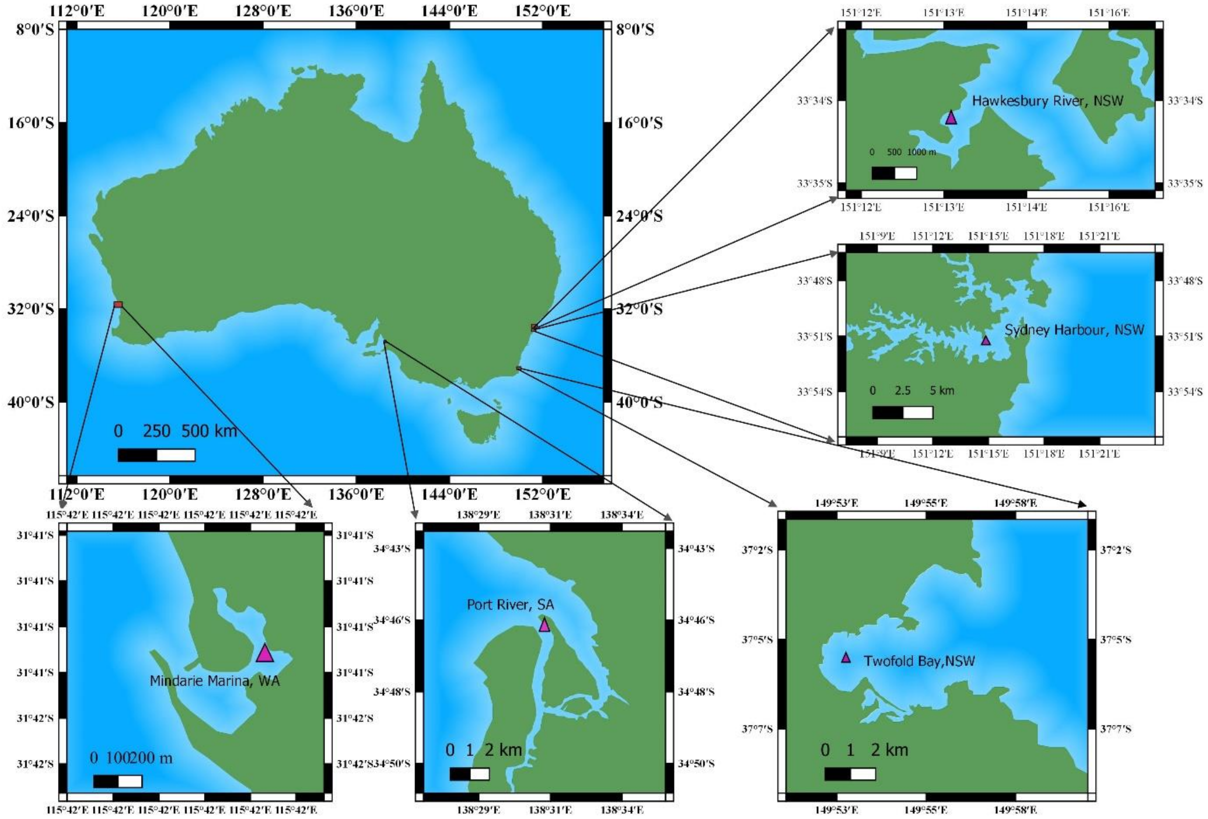


Figure 4.2: Sampling site locations for all strains established in this study.

4.3.2 Molecular identification of dinoflagellate strains

Approximately 50 ml of culture of each strain was harvested by centrifugation (5 min at 2500 rpm). These cell pellets were used for DNA extraction using the FastDNA spin kit for soil (MP Biomedicals, Solon, OH). The extracted DNA quality was checked using a Nanodrop (Nanodrop 2000; Thermo Scientific, Australia). DNA amplification of D1/D3 region of the

LSU rRNA gene was performed for all of the strains. All PCR reactions were performed in a reaction volume of 25 µl containing 5 µl of 5X MyTaq reaction buffer (Bioline, Sydney, Australia), MyTaq DNA polymerase (Bioline, Sydney, Australia) 0.5 µl, 7.5 pmol of each primer, 1 µg μl^{-1} of BSA (Biolabs, Arundel, Australia), 1 µl of template DNA and 15.5 µl of PCR grade water using previously published primers: D1F (Scholin et al. 1996) and D3B (Nunn et al. 1996). The thermocycling conditions were performed using the following steps: an initial denaturation step of 94°C for 5 min, followed by 35 cycles of 94°C for 30 s, 56°C for 30s, and 72°C for 1 min, followed by a final extension step of 3 min. 1% agarose gel stained with GelRed (Gene target solutions, Dural, Australia) was used to verify the amplification. PCR product purification was performed with Zymoclean™ gel DNA recovery kit (Zymo Research, Irvine, CA), following the supplied protocol from the manufacturer. Sequencing was performed using the commercial service of Macrogen (Korea).

4.3.3 Phylogenetic tree inference based on the LSU sequences of the strains

Detail method of phylogenetic tree has been described in chapter 2

4.3.4 RNA extraction, library preparation and sequencing

Approximately 10^5 cells of all of the strains were harvested by slow centrifugation (at 2300g for 10 min) during their exponential phase of growth. The cell pellets were washed with 1x PBS (Phosphate Buffer Saline) (Sigma, Australia) to remove the aggregated bacterial cells and sea water crystals. Snap freezing of pellets in liquid nitrogen was performed immediately after the PBS wash. The strains were stored in -80°C before the final RNA extraction.

During the RNA extraction 1 ml of ice-cold TRI reagent (Life Technologies Australia Pty Ltd) was added in the cell pellet and allowed to heat at 60°C for 2 minutes. This mixture was taken for beat beating at a top speed for 1 min. An aliquot of 200 µl of chloroform was added into the collected supernatant after bead beating. Centrifugation was performed for 10 min at 12000g at 4°C. The top aqueous layer was separated and mixed with 1x volume of isopropanol. This was stored at -80°C for 2 hours before further processing. Purification steps were performed using RNeasy Mini Kit (Qiagen Pty Ltd) according to the supplier's guidelines. RNA was stored at −80°C after extraction. The RNA purity, quantity and integrity were assessed using a Nanodrop ND-1000 (Thermo Scientific, Woltham, MA) and 2100 Bioanalyser

(Agilent Technologies, Santa Clara, CA). The libraries were prepared using TruSeq stranded mRNA sample prep following the manufacturer's instructions and sequencing was performed using NovaSeq 6000 2 × 150 base pair (bp) paired end reads.

4.3.5 Assembly

4.3.5.1 Reference transcriptome preparation

The reference transcriptome of strain CS798 was assembled using Trinity vs2.8.4 (Grabherr et al. 2011) with parameters -trimmomatic for read trimming with default parameters and --RF as the RNASeq library was prepared with TruSeq stranded libraries.

To create a set of high-confidence transcripts, we first predicted open reading frames (ORFs) using Transdecoder v5.5 (https://github.com/TransDecoder) with default parameters. The resulting protein sequences were compared to known proteins in the Uniprot SwissProt database (Bairoch & Apweiler 1996) and transcripts were filtered out if (a) predicted proteins were shorter than 80% or longer than 120% of a known SWISS-Prot sequence, or (b) if the protein sequence identity of the predicted peptide was <50%. This resulted in a filtered reference set of 5,216 assembled transcripts ranging from 299 - 1,240 nucleotides in length.

4.3.5.2 Assembly of sequences

RNA reads of each sample were assembled independently using Trinity v2.8 (Grabherr et al. 2011). Prior to assembly reads were trimmed with Trimmomatic (Bolger, Lohse & Usadel 2014) using Trinities –trimmomatic option. Assembly statistics were computed using the stats.sh script included in BBMap (Bushnell 2014).

4.3.6 SNP calling and analyses

4.3.6.1 SNP calling using GATK

Prediction of single-nucleotide polymorphisms (SNPs) using the Genome Annotation Tool Kit (GATK) v4.1.9 (McKenna et al. 2010) based on the GATK's Best Practice for RNASeq data. In summary, reads of each individual strain were mapped to the filtered reference transcriptome using the splice-aware aligner STAR v2.7.6a (Dobin et al. 2013) and converted to sorted bam files using Samtools v1.10 (Li et al. 2009). Using PicardCommandLine, which is included in the GATK package read groups, were then added to each individual strain's alignment file, duplicates were marked and alignments of reads including "N" were split. The resulting bam file was used to predict SNPs using GATK's HaplotypeCaller with default parameters.

4.3.6.2 SNP calling using Freebayes

SNP prediction with Freebayes v1.3.2 (Garrison & Marth 2012) was performed on the bam files, with added read groups and marked duplicates using PicardCommandLine (see SNP calling using GATK for details). SNP calling was then performed using Freebayes with default parameters and option --gvcf to create a genome wide variant file.

4.3.6.3 SNP calling using bcftools

SNPs were predicted using bcftools v1.10.2 (Narasimhan et al. 2016) by first creating coverage profiles of the duplication marked bam files for each strain (see SNP calling using GATK for details) using samtool's mpileup command. Variants were called subsequently using bcftools call command with default parameters.

4.3.6.4 SNP extraction and filtering

Genome vcf files were further processed using bcftools v1.10.2 (Narasimhan et al. 2016). SNP positions were extracted from each gvcf file using the filter command with options -i 'TYPE="snp"' to create SNP only vcf files for each strain and SNP caller. To create a set of highly supported SNP positions for each strain subsequent intersection of the vcf files was performed using bcftool's isec command. Subsequent filtering of the intersected variant set was with thresholds recommended by GATK (MQ>40, QD<2, ReadPosRankSum<0.8, FS>40, SOR>3) and performed with bcftools' filter command resulted in no further variants filtered from the intersections (see filtered and unfiltered SNP statistics below). Finally, the resulting filtered high-confidence SNP vcf files were merged using bcftools merge command.

4.3.6.5 Clustering analysis

Genetic distance was calculated at the beginning using the required command lines in R 3.6. Based on distance data hierarchical clustering was performed using package “hclust”.

4.3.7 Toxin analyses

Toxin extractions were performed using the method of Harwood et al. (2013)(Harwood et al. 2013). Briefly, five millilitres of 1 mM acetic was added to each sample tube and vortexed for 90 secs. The sample tubes were placed into boiling water bath for 10 min, followed by cooling at room temperature. The samples were then placed in an ultrasonic bath for 1 min. Finally sample tubes were centrifuged for 5 min to separate the cell debris. The supernatant was used for chromatographic separation on a Thermo Scientific™ ACCELA™ UPLC system (either with or without dilution).

A Thermo Scientific™ Q EXACTIVE™ high resolution mass-spectrometer equipped with an electrospray ionization source was used for the analysis. The following source parameters were used in all experiments: a capillary temperature of 263 °C, a spray voltage of 3.5 kV, an auxiliary gas heater temperature of 425 °C, a sheath gas and an auxiliary gas flow rate of 50 and 13 (arbitrary units). The mass spectrometer was operated in polarity switching mode scanning across the range of m/z 100–500. LC-MS was performed on a Thermo Scientific™ ACCELA™ UPLC system by the method published by Boundy et al. (2015). Separation was carried out by an Acquity UPLC BEH Amide 130 (150 mm × 2.1 mm i.d., 1.7 µm particle size) column. Mobile phases were A. Water: Formic acid: NH4OH (500:0.075:0.3) and B. Acetonitrile:Water:Formic Acid (700:300:0.1). The injected volume was 5 µL. Thermo Xcalibur software (version 3.0.63, Thermo Fisher Scientific, Inc.) was used for the data analysis. Analytical standards for the PST analogues were obtained from National Research Council, Canada.

4.4 Results

4.4.1 Phylogenetic analysis of the *Alexandrium* strains

Phylogenetic analysis of all the *Alexandrium* strains, using Maximum Likelihood and Bayesian Inference conducted on the LSU rRNA (D1/D3) regions show that they cluster together with other *Alexandrium pacificum* strains with full support (Figure 4.2, Bayesian PP = 1, ML BS = 100).

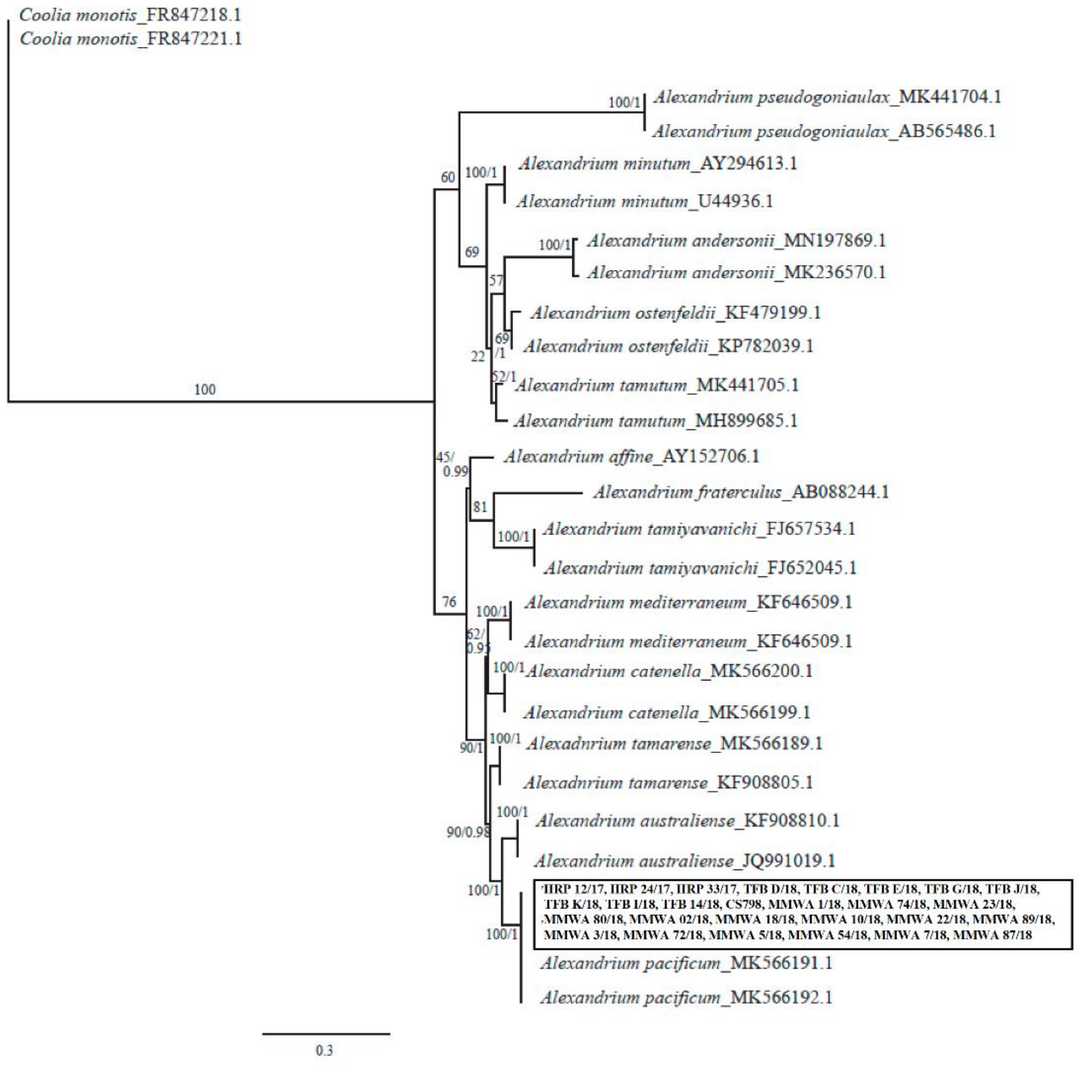


Figure 4.3: Phylogenetic tree based on large subunit (LSU) rRNA. of *Alexandrium*. The trees were constructed with Bayesian Inference (MrBayes). Numbers at nodes represent posterior probabilities from Bayesian Inferences (BI) and bootstrap support values from Maximum Likelihood (ML) based on 1000 replicates. (A)

4.4.2 Overview of the transcriptome sequencing

Transcriptome sequencing of *Alexandrium pacificum* strains were performed by Illumina NovaSeq 6000 2 × 150 base pair (bp) paired end reads and were assembled using Trinity vs2.8.4. Table 4.2 shows the number of assembled transcripts, GC(%) and GC_stdev. The assembled transcript number varied within a range of 120000 to 190000. The GC dinucleotide content was greater than 56%.

Table 4.2: Overview of the transcriptome sequencing

Strain ID	Number of assembled transcripts	GC (%)	GC(standard deviation)
TFB_D_18	163839	58.79	0.0515
TFB_J_18	165660	57.93	0.0561
TFB_K_18	175330	57.31	0.0551
TFB_I_18	167212	58.68	0.0524
TFB_14_18	182054	58.12	0.0706
TFB_E_18	159938	59.00	0.0594
TFB_C_18	159860	58.44	0.0587
TFB_G_18	149137	58.64	0.0614
HRP_12_17	149681	58.42	0.0504
HRP_24_17	120002	56.97	0.0526
HRP_33_17	124455	57.25	0.0562
ASCH02	148286	59.62	0.0519
CS798	174059	59.00	0.0585
MMWA_1_18	164345	59.48	0.0558
MMWA_3_18	187354	59.82	0.0617
MMWA_5_18	171744	58.12	0.0598
MMWA_7_18	169003	58.32	0.0511
MMWA_10_18	158709	57.69	0.0519
MMWA_18_18	165540	57.03	0.0645
MMWA_22_18	138655	57.35	0.0595
MMWA_23_18	158692	58.28	0.059
MMWA_54_18	165772	58.87	0.0632
MMWA_72_18	176898	59.41	0.0589
MMWA_74_18	160399	58.04	0.0687
MMWA_80_18	171123	58.04	0.0687
MMWA_87_18	182959	57.72	0.0574
MMWA_89_18	156347	57.55	0.0596

4.4.3 Single nucleotide polymorphism (SNP) prediction

Three different SNP callers, GATK, Freebayes and bcftools were used to predict the SNPs against the reference strain CS798. Figure 4.3 shows the SNP counts of strains using all three different SNP calling methods (GATK, Freebayes, bcftools). The highest number of SNPs was identified using Freebayes in all of the strains (~11000 to 47000, Figure 4.4), the lowest number of SNPs was detected using the bcftools (~9000 to 20000, Figure 4.4), and the number of SNPs detected by GATK was in within the range ~9000 to ~29000 (Figure 4.4). The lowest number of SNPs were detected in the strain ASCHO2 using all three different callers.

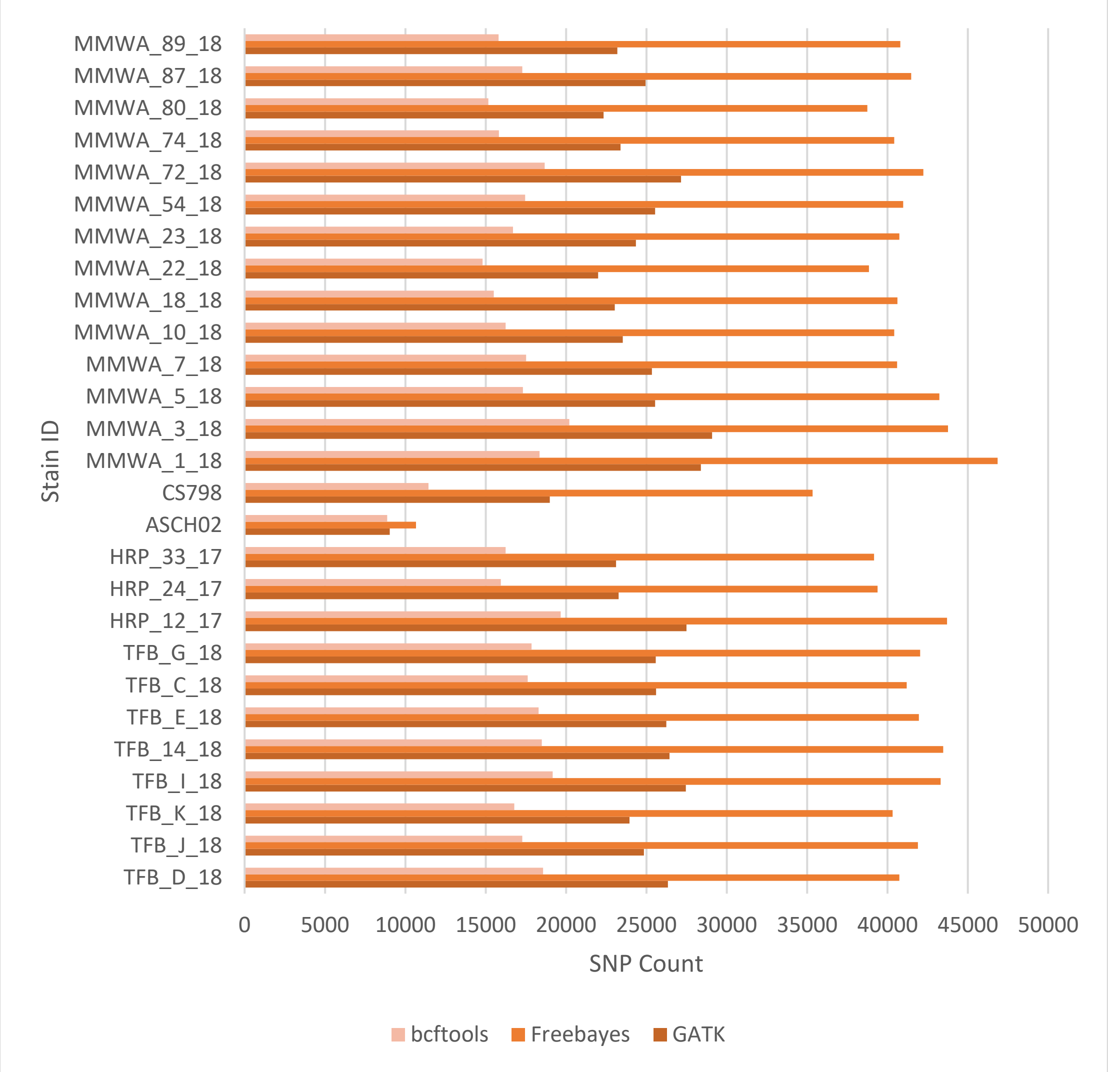


Figure 4.4: Single Nucleotide Polymorphisms (SNPs) identified in *Alexandrium pacificum* strains using three different caller GATK, freebayes, and bcftools

4.4.4 Genetic diversity

Hierarchical clustering was performed based on the genetic distance of the identified SNPs. Four clusters were identified in the analyses (Figure 4.5). All of the strains isolated from Mindarie Marina, Western Australia (strain ID's started with MMWA Figure 4.5 A) have been identified to cluster in one group (Figure 4.5B). Strain CS798, which was isolated from Port River, South Australia, was grouped in the same cluster (Figure 4.5B). The strains isolated from Twofold Bay, New South Wales (Strain ID started with TFB Figure 4.5A) were grouped in another cluster (Figure 4.5B). Similarly, the strains isolated from Hawkesbury River, NSW were identified in another separated cluster (Figure 4.5B). The Sydney Harbour strain ACSHO2 was not closely related to any other strain identified in this study (Figure 4.5B).

Three different sub-clusters were observed in the cluster of MMWA strains and CS798 which was comprised of 16 strains (Figure 4.5B). Two different sub-clusters were identified in the TFB cluster, the cluster of 8 strains (Figure 4.5B). A nucleotide divergence of 1.0 was identified for the strain ASCHO2, which indicates a high genetic diversity of this strain compare to the others (Figure 4.5B). For the HRP strains (isolated from Hawkesbury River) the nucleotide divergence was 0.5 and the TFB strains showed a genetic divergence of ~ 0.65 (Figure 4.5B).

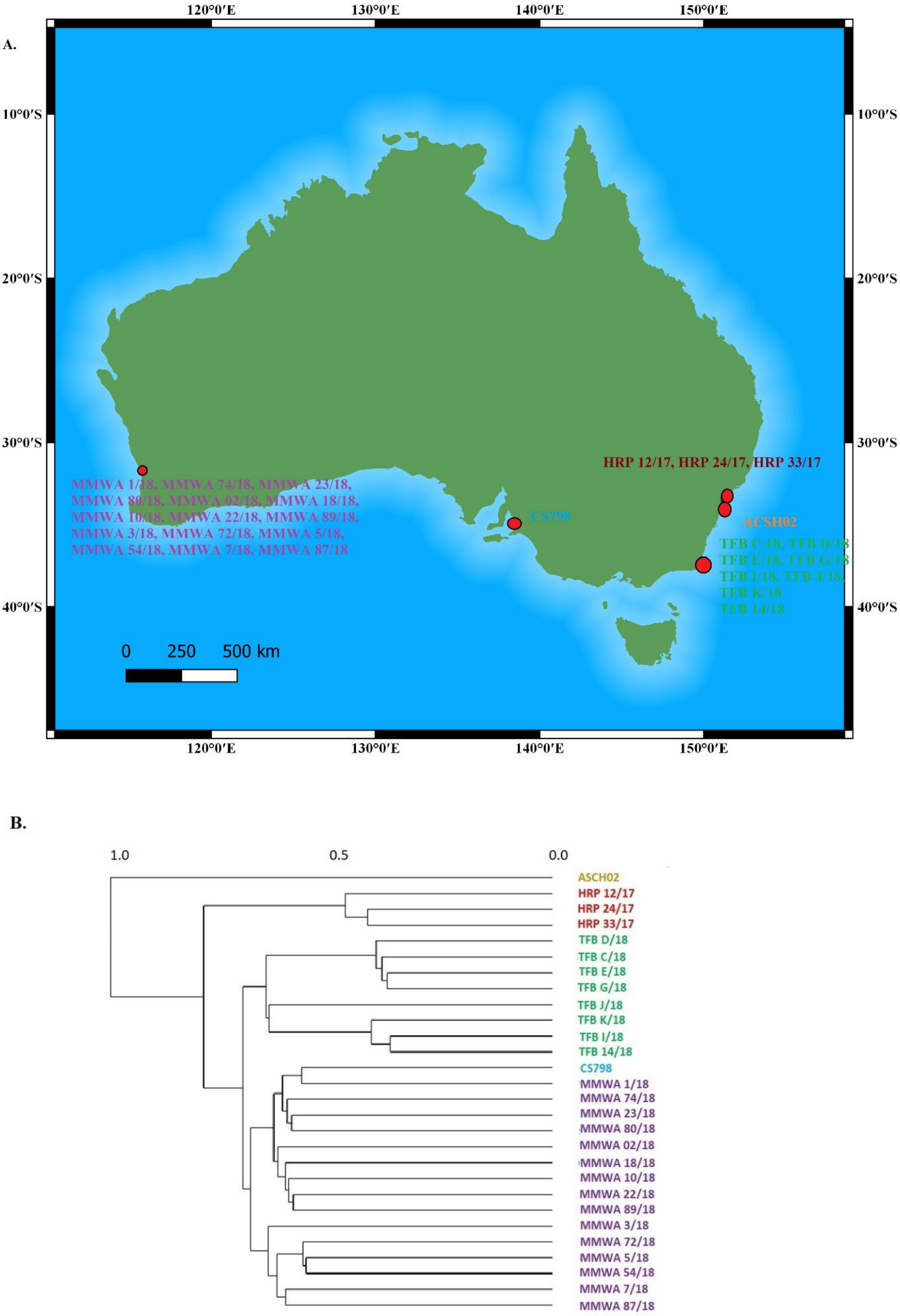

A.
120°0′E
130°0′E
140°0′E
150°0′E
10°0′S
20°0′S
30°0′S
40°0′S
HRP 12/17, HRP 24/17, HRP 33/17
MMWA 1/18, MMWA 74/18, MMWA 23/18,
MMWA 80/18, MMWA 02/18, MMWA 18/18,
MMWA 10/18, MMWA 22/18, MMWA 89/18,
MMWA 3/18, MMWA 72/18, MMWA 5/18,
MMWA 54/18, MMWA 7/18, MMWA 87/18
CS798
ACSH02
TFB C/18, TFB D/18
TFB E/18, TFB G/18
TFB I/18, TFB J/18,
TFB K/18
TFB 14/18
0 250 500 km
B.
1.0
0.5
0.0
ASCH02
HRP 12/17
HRP 24/17
HRP 33/17
TFB D/18
TFB C/18
TFB E/18
TFB G/18
TFB J/18
TFB K/18
TFB I/18
TFB 14/18
CS798
MMWA 1/18
MMWA 74/18
MMWA 23/18
MMWA 80/18
MMWA 02/18
MMWA 18/18
MMWA 10/18
MMWA 22/18
MMWA 89/18
MMWA 3/18
MMWA 72/18
MMWA 5/18
MMWA 54/18
MMWA 7/18
MMWA 87/18

Figure 4.5: Genetic diversity in *Alexandrium pacificum* strains in Australia. A. The isolation sites of *Alexandrium* pacificum strains B. Hierarchical clustering of the strains with genetic distance.

4.4.5 PST profile of *Alexandrium pacificum* strains

The LC-MS results confirmed the presence of PSTs in all of the strains except CS798, which is known to be an anomalous non-PST producing strain (culture established from cyst germination). Strains were examined to identify 14 STX analogues C1, C2, GTX1-6, dcGTX2-3, dc STX, STX, Neo, and dcNeo. The strains showed variation in their toxin profile (Figure 4.6) GTX 4, 5, 6, and C2 were observed to be present in high quantity in the strains isolated from Mindarie Marina (MMWA strains). The abundance of C1 and C2 analogues were observed in the isolates from Hawkesbury River. The toxin profile of the strains isolated from Twofold Bay, NSW showed the presence of GTX1, GTX4 and GTX5 in higher concentration compare to the other analogues (Figure 4.6).

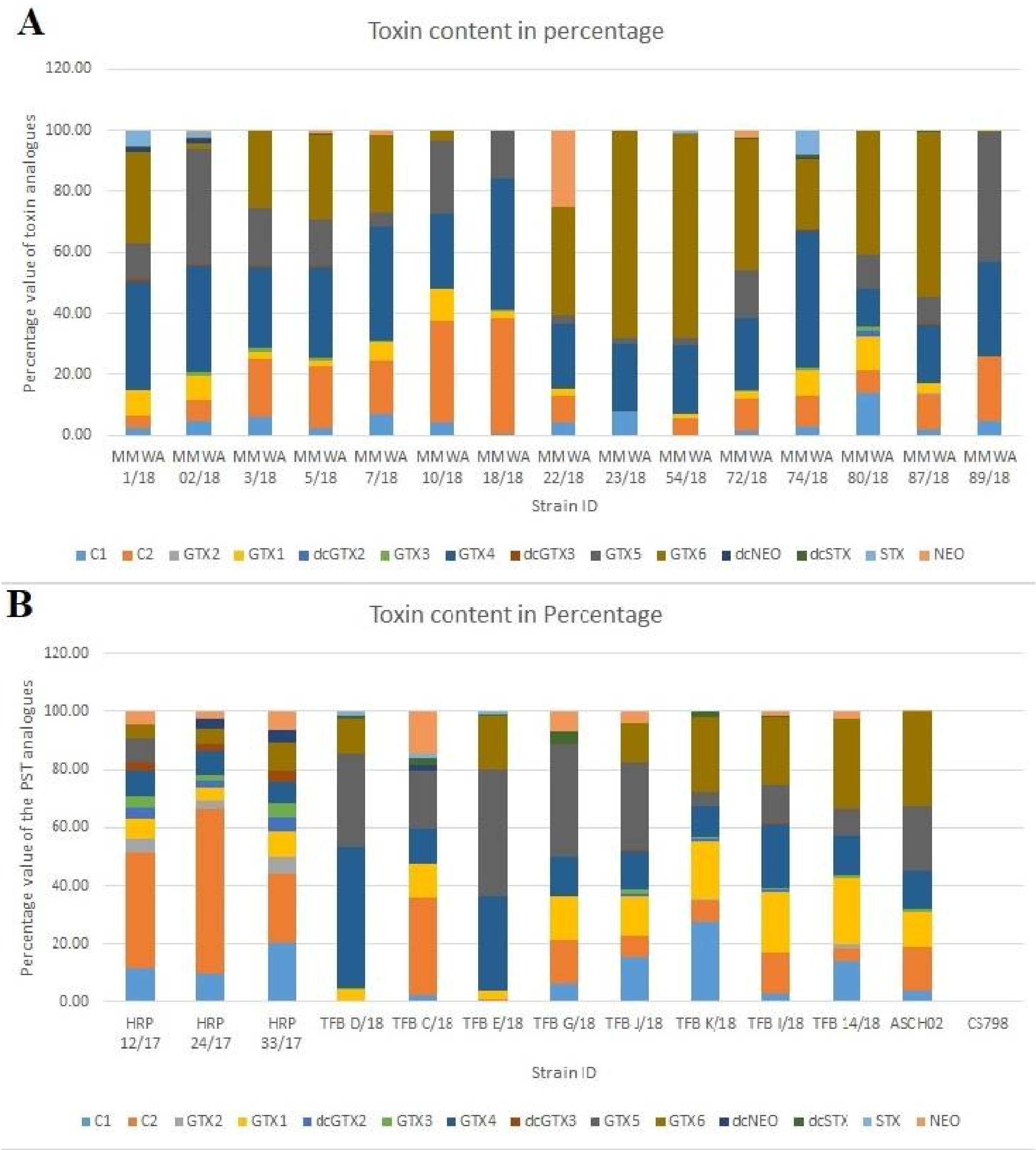


Figure 4.6 : Percentage of the PST analogues in different *Alexandrium pacificum* strains

4.5 Discussion

4.5.1 Genetic differentiation of *Alexandrium pacificum* population

In this work, the relationship among 28 strains of the PST producing species *Alexandrium pacificum* from the eastern, southern and western coasts of mainland Australia, covering an area of ~ 4000 km, was examined. PSTs from *Alexandrium* species, and *Alexandrium pacificum* in particular, have led to PST uptake above the regulatory level in aquaculture shellfish in south-eastern Australia and the closures of shellfish farming for periods of months (Barua et al. 2020). Blooms of *Alexandrium pacificum* in Western Australia have also occurred recently (this study, (Dias et al. 2015). For the past ~20 years, it was known that *Alexandrium pacificum* was present in coastal and estuarine waters of South Australia, Victoria, Tasmania and New South Wales (Bolch & de Salas 2007; de Salas et al. 2001; Farrell et al. 2013; Hallegraeff 1992; Hallegraeff et al. 1991; Murray et al. 2011). It has been speculated that *Alexandrium pacificum,* identified in Western Australia in 2015 for the first time, was likely present there as a result of a bioinvasion, possibly due to ballast water discharge (Dias et al. 2015). As shellfish aquaculture is a rapidly growing industry in Australia particularly in Western Australia, it is important to understand the relationship among strains of *Alexandrium pacificum* from different Australian regions, to determine whether they likely represent recently introduced populations, and if so from where, and to determine the impact their diversity has on their PSTs produced.

Initially, it was considered that most marine phytoplankton show a cosmopolitan distribution, have little or no barriers to gene flow in the environment, and reproduce rapidly, and consequently, little genetic structuring would be present amongst phytoplankton populations from different ocean regions (Foissner 2007; Medlin, Sáez & Young 2008). However, studies that have applied genetic techniques such as microsatellites, and more recently SNPs or RADseq to understand population genetics among phytoplankton species, have found that extensive local genetic structuring on both a temporal and spatial scale can exist (Casabianca et al. 2012; Gao et al. 2019; Godhe et al. 2016). Populations of marine phytoplankton which have a dormant cyst stage in their life may be even more likely to exhibit local endemism, as cysts may be retained in the benthos and be less likely to be transported (Sundqvist et al. 2018). *Alexandrium* species have the capacity to form resting cysts, and it has been reported that *A. pacificum* can survive in this condition over 100 years (Anderson 1998; Mizushima &

Matsuoka 2004). Resting stage formation in the life cycle is considered as the reservoir of genetic information when the cells are reported to be resistant against the genetic drift. Therefore, there was a possibility that establishment of cyst might be linked to show the genetic differentiation and hence show a high degree of endemism in the strains isolated from sites of two different boundary current EAC and LC in Australia.

In our study, we have identified that the *A pacificum* strains showed genetic structuring (figure 4.4) and that strains isolated from the Leeuwin current (LC) region formed a distinctive cluster from strains isolated from the locations of the East Australian Current (EAC). These two different boundary currents, the East Australian Current (EAC) and the Leeuwin Current (LC), both flow from the north to south, and fringe the coastline of Australia (Figure 4.1). Boundary currents in the ocean can strongly influence the population connectivity and can cause spatial heterogeneity (Coleman et al. 2013). Local and regional oceanography facilitates the dispersal of marine organisms, controlling the connectivity (exchange of genetic material) within and among populations, and thus influencing the ecology and evolution of a region (Coleman, Chambers, et al. 2011). The East Australian Current (EAC) is the dominant western boundary current on the east coast of Australia, originating in the tropical Coral Sea and flowing south along the edge of the continental shelf (Figure 4.1). The Leeuwin Current, on the other hand is a warm current which flows southwards of the coast of Australia These currents are important in the transport of more tropical waters into southern latitudes. There is also evidence that the EAC and LC strongly impact the distribution of marine organisms (eg. sea urchins and kelp), yet their influence on the connectivity of marine phytoplankton populations along these east and west coastlines is to date unknown (Banks et al. 2007; Coleman, Roughan, et al. 2011).

In one particular study, genetic differentiation was identified in a geographically isolated strain of *A. pacificum*, which may indicate that oceanic currents and geographic boundaries may be the limiting factors to a shared and common gene pool (Genovesi et al. 2015). A similar study was performed using *A. minutum* strains in the Mediterranean area, where the clustering of the strains based on the geographical location was not identified because of unbalanced sampling (Le Gac et al. 2016).

Species of *Alexandrium* show a haplo-diplophasic life cycle, which means they have both a sexual and an asexual phase of life (Anderson 1998). High genetic diversity has been reported in the strains isolated from cysts and vegetative cells of *A catenella* (Alpermann et al. 2010;

Erdner et al. 2011; Paredes et al. 2019). Mutation can cause genetic variation in during the asexual phase of life when they proliferate in the water column and when the genetic recombination happens during mating (Mardones et al. 2016). The newly introduced cyst can sink in the sea sediment, mix with the old cyst and act as a genetically differentiated seed bank for that particular location (Godhe & Harntrom 2010). In this way, the dormant stage of dinoflagellates can increase the adaptability in a particular locality (Sundqvist et al. 2018). During environmental change, the seed bank can display differently adapted alleles that may enhance genetic diversity in a similar way to plants (McCue & Holtsford 1998).

In the current study, the strain of *Alexandrium pacificum* from South Australia isolated in 1993, clustered together with strains from Mindarie Marina in Western Australia, which are also part of the Leeuwin Current system. This indicates that it likely that the *Alexandrium pacificum* population in Western Australia represents a long resident population, that likely went undetected for many years, or possibly an introduced population from South Australia. It has previously been found that a 'rare biosphere' of undetected species can exist in regions (Lynch & Neufeld 2015). Furthermore, the results of this study do not support this *Alexandrium pacificum* population as being introduced from other locations such as eastern Australia (Dias et al. 2015). It has been hypothesized that ballast water introduced the *Alexandrium* cells in Australia from Japan (Bolch & de Salas 2007; Hallegraeff & Bolch 1992). Whereas *A. pacificum* strains from EAC are genetically different from the LC strains, more research on the genetic similarities of Japanese isolates with the Australian strains is needed to confirm this hypothesis.

It has been reported that the long-term maintenance of dinoflagellate culture in the laboratory may lead to selective pressure may induce genetic changes in strains that influence population genetic studies (Lakeman, von Dassow & Cattolico 2009). In our study, we used strain CS798 that was isolated in South Australia in 1993 as a reference strain, and clustered with high support with strains recently isolated from Western Australia (Figure 4.5). This suggests that the mutation rates of dinoflagellate strains in long term culture may be slower than has been speculated, and in our study is unlikely to have influenced the inference of population genetic structuring.

4.5.2 Variation in SNP counts depending on variant caller

Various studies have found that the number of identified SNPs can vary depending on the use of different variant callers (Ni et al. 2015; Schilbert, Rempel & Pucker 2020; Zhao et al. 2019). Here, we found that GATK and SAMtools (bcftools) were both more appropriate statistical methods than freebayes in terms of SNP variant calling. GATK has been reported to give slightly higher counts than bcftools, as bcftools are more stringent about SNV (single nucleotide variation), whereas GATK considers INDELs (insertion and deletions) simultaneously (Schilbert, Rempel & Pucker 2020). Although all of the variant callers use Bayesian methods to detect variants, Freebayes can produce the highest count, as it does not use a precise alignment like GATK and bcftools, but uses the literal sequences of reads aligned to a particular target (Ni et al. 2015).

4.5.3 Toxin profile of *Alexandrium pacificum* strains

The toxin profile of different strains of *A. pacificum* isolated from the same location in this study showed variation in the analogues that are present in the different strains, along with differences in the percentage of toxin content. No pattern of PST profile was detected between the eastern and western strains. Similarly *A. pacificum* strains isolated from south western Mediterranean marine ecosystem, Algeria also showed this high variability in toxin profile and content (Hadjadji et al. 2020). To understand the relation between the difference in the toxin content and genetic variation, more studies are required where SNP variability and *sxt* genes could be examined across multiple strains and locations.

In conclusion, understanding the genetic structure and toxicity of *A. pacificum* is important in order to understand the evolutionary trajectory *of Alexandrium pacificum* and investigate the origin of population's in particular geographical locations in Australia. Investigations into the ecological triggers of *Alexandrium pacificum* HABs in particular locations provide information help to monitor and predict future HABs of this species in this region. The information also assists us in understanding the potential for *Alexandrium pacificum* to be affected by environmental changes and its potential for local adaptation.

Chapter 5:

Thesis Discussion

5.1 Overview

The genus *Alexandrium* is considered as the most widely studied dinoflagellate species due to their HAB forming capacity and potential neurotoxin PST producing capacity (Anderson et al 2012, Murray et al 2015). Saxitoxin and its derivates are the members of this neurotoxin group which can be synthesized by a group of *sxt* genes (Kellmann et al, 2010). Despite of having a complex genomic structure like other dinoflagellates, the advancement in the genomics and transcriptomics helped to identify some possible ways of gene regulation and to characterize the genes that can control the PST biosynthesis pathway, though it is still not clear. In order to control the impact of the PST producing *Alexandrium* bloom and to make advancement in monitoring by early warning system development, it is important to have precise knowledge on *sxt* genes and their regulation.

Alexandrium has the capacity to produce resting cysts in their life cycle which facilitate the best chances of adaptation in unfavourable environment. It is also widely believed that the resting cyst may help to distribute them worldwide by the introduction of ballast water. This cyst may have the significant role in the genetic differentiation of *Alexandrium.* In Australia, the *Alexandrium* bloom has become too frequent now-a-days both in the global climate change hotspot EAC and in LC. Understanding the population genetic structure of *Alexandrium* in Australian coastal water will give the opportunities to understand the evolution of this marine algae with the information on the previous demographic origin. This information might be helpful for future bloom and the intensity prediction in response to the climate change.

5.2 Significance of the study

5.2.1 First detection of *Alexandrium pacificum* associated PSTs above the regulatory limit in New South Wales

In the first chapter, a detail study was undertaken to investigate an unprecedented PST event that was happened in Twofold Bay, New South Wales, Australia in 2016. This was the first record of PSTs produced by *Alexandrium pacificum* above the regulatory limit 0.8 mg/Kg in the commercial aquaculture area in south-eastern Australia. This bloom was described as an intense event as it persisted for at least eight weeks with a highest cell concentration of 89,000 cells L^{-1} of *A. pacificum* and maximum reported PSTs concentration in mussel tissue was 7.2

mg/Kg STX equivalent PST. During that time, *A. pacificum* cells were detected up to 13 km north and 21 km south along the adjacent coastline (NSWFA 2017). Before this event, the highest recorded PST detection in NSW was 0.66 mg/Kg which was below the regulatory limit (NSWFA 2017). During the massive Tasmanian PST event in 2012 when a worldwide product recall was happened, the highest detected PST concentration was ~10 mg/Kg (Bolch et al 2014). At the time of the study period, the identity of *A. pacificum* was confirmed using molecular genetic tools (qPCR and amplicon sequencing) and complemented by light and scanning electron microscopy of cultured strains. The follow up study was continued in the next two years as subsequent blooms of *A. pacificum* occurred in Twofold Bay in 2017 and 2018. The presence of cryptic biodiversity is evident in marine planktonic population. This kind of cryptic biodiversity could possibly be responsible to lead this massive bloom. Detail population genetic study was performed in Chapter 4 where eight *A. pacificum* isolates were used that were established from Twofold Bay.

During this study, the identity of the species, its toxicity and the environmental conditions at the time of this shellfish contamination were examined. Environmental factors may significantly contribute to the initiation, termination and the intensity of harmful algal blooms (HABs) which may include elevated nutrient levels, availability of micronutrients, water stratification, presence of copepods and seeding from benthic cysts during favourable conditions (Cembella et al 2003, Johen & Flynn 2002, Juhl et al 2001, Grzebyk et al 2003, Sundqvist et al 2018). The availability of inorganic nitrogen and inorganic phosphorus can shift the eukaryotic community composition in oceans from a dinoflagellate dominated community to one of diatoms (Zhou et al 2018). For this reason, in Chapter 3, we have investigated the influence of copepod to increase the level of PST production using *Parvocalanus crassirostris* which is common in Australian coastal water. In our investigation, we have been identified a flow of unusually cool and nutrient rich water into Twofold Bay during that bloom time in 2016 which may have a significant contribution to the unprecedented growth of this taxa.

The presence of *Alexandrium* cysts have already been reported in the major shipping port of Eden (Twofold Bay) (NSWFA 2017). Moreover, following the initial detection of *A. pacificum* in 2016 in Twofold Bay, a high abundance of this species was observed in the next two years though the severity was less compared to the bloom of year 2016. These repeated blooms confirm the establishment of a cyst bed in this region. The knowledge from this study will be helpful to predict the future bloom intensity in other locations where closely related *A. pacificum* blooms are very common. A detail investigation on the population genetic structure of *A. pacificum* isolated from Twofold Bay, NSW was performed in Chapter 4 to get more information on *A. pacificum* responsible for that unprecedented bloom.

5.2.2 Molecular mechanism of saxitoxin biosynthesis

As PSTs are considered amongst the most potent neurotoxins naturally produced, several studies have been carried out to understand gene regulation of PST producing genes in dinoflagellates (Murray et al. 2012; Stüken et al. 2011; Suikkanen et al. 2013; Wiese et al. 2014; Zhang et al. 2014). In Chapter 3, I undertook a detailed comparative study between PST producing and PST non-producing *Alexandrium pacificum* strains using an assay of ~8 PST related genes (sxt) and several housekeeping genes using NanoString technology. In this study, I exposed *Alexandrium pacificum* strains to a copepod species, *Parvocalanus crassirostris,* which was found to produce copepodamide. It has been reported that the presence of copepodamide may influence the defence system of *Alexandrium species* (Selander et al. 2015). For this research, I used two anomalous non-PST producing strain of *Alexandrium pacificum*, both of which were originally isolated from cysts. I compared gene expression in these two strains with expression in two high PST producing strains of *Alexandrium pacificum* in the presence and absence of copepod cues.

The results confirmed the involvement of *sxtA* as the main gene in PST biosynthesis (Murray et al. 2015; Stüken et al. 2011; Zhang et al. 2014). A significantly higher transcript abundance was found of the long isoform of *sxtA* which includes the domain *sxtA4*, one of the multiple isoforms of *sxtA1* (Accession no JF343240.1) and the domain *sxtA4,* were confirmed in the two PST producing *A. pacificum* strains in our study. A 48 hour copepod exposure of PST producing *A. pacificum* strains significantly increased the PST production in *Alexandrium pacificum* strains, however this difference was not reflected in a significantly higher level of transcription in the *A. pacificum* strains. This result confirmed that the PST biosynthesis pathway appears to be generally regulated at the post-transcriptional level.

Many studies have confirmed the absence of the gene *sxtA4* in non-PST producing dinoflagellates (John et al. 2014; Murray et al. 2015; Orr et al. 2013b; Stüken et al. 2011). The exception to this pattern appears in the genome of the anomalous non-toxic strains of *A. pacificum* that were used in this study, and some reported strains of *A. minutum,* where the domain domain *sxtA4* is still present (Zhang et al. 2014). In a previous study, the expression of *sxtA4* in a non-toxic mutant strain of *A. pacificum* was not observed (Zhang et al. 2014). Here, we identified the presence of an *sxtA4* transcript in the non-PST producing *Alexandrium pacificum*, but at a significantly lower level compared to the PST producing strains.

5.2.3 Genetic differentiation of *Alexandrium pacificum* population in Australia

In chapter 4, I used *Alexandrium pacificum* strains from the eastern, southern and western coasts of mainland Australia to understand the population genetic structure of this species. This is the first time that a study has been conducted on population genetic structure of a phytoplankton species in Australian coastal waters. It was identified that the stains isolated from Western Australia and South Australia, as part of the Leuwin current system, distinctly clustered as one group, and were clearly separated from the strains isolated from the East Australian Current. This result contradicts a previous claim that *Alexandrium pacificum* was likely recently introduced to Western Australia, possibly due to ballast water discharge, and probably from the eastern states of Australia, where its presence has been known for some time (Dias et al. 2015).

Population genetic studies of species of *Alexandrium* have confirmed the presence of both temporal and spatial scale genetic diversity in other regions in the world (Casabianca et al. 2012; Gao et al. 2019; Godhe et al. 2016). In this study, I identified the presence of genetic structuring in *A. pacificum* strains isolated from two different boundary currents (EAC and LC) in Australian waters. Populations of *Alexandrium* species may be more likely to show local endemism (Sundqvist et al. 2018) due to the presence of a resting cyst in its life cycle. In this study however, the PST toxins of isolates from different locations around the Australian coast did not show any significant pattern.

5.3 Future direction

The EAC is considered to be a global hotspot of climate change, as faster warming and a more southerly flow of the EAC has been observed in this area (Frusher et al. 2014). The frequency of HABs due to *Alexandrium pacificum* and *Alexandrium catenella* impacting shellfish aquaculture have increased since 2012 in this region (Farrell, Ajani, et al. 2014; Hallegraeff et al. 2020), and now occur on a regular basis. In the past 5 years, the presence of *Alexandrium pacificum* in Western Australian waters has been confirmed for the first time. More studies are needed to understand whether the warming and increased southward flow of the EAC is associated with the presence and abundance of *Alexandrium pacificum* in this region, or whether better detection technologies, improved shellfish safety monitoring and increases in shellfish aquaculture are the cause of this apparent increase.

In this work, it was confirmed that PST biosynthesis is strongly related to the expression of certain *sxt* genes in particular, including *sxtA4* in non-PST producing strains of *Alexandrium pacificum*. We provide evidence that this gene may be regulated at a post-transcriptional stage in this species. Further studies investigating the translational or post translational stage in PST biosynthesis in dinoflagellates are required to understand *sxt* gene regulation. Moreover, a more elaborate study could be performed using different copepod species along with different toxic and non-toxic *Alexandrium* species, to understand the role of mixed copepods in the toxin profile and gene regulation. This understanding could be used to understand the role of the presence of copepods in *Alexandrium* bloom formation, with the outcome potentially used in bloom mitigation into the future.

Using isolates of *A. pacificum* from the eastern, southern and western coasts of mainland Australia, we have found that strains cluster based on the current system of their isolation site, and that it appears unlikely that the Western Australian strains represent a recent introduction or bioinvasion. It has been hypothesised that Australian strains of *Alexandrium pacificum* and *A. catenella* originated as ballast water imports from Japanese coastal areas, due to international shipping (Bolch & de Salas 2007; Hallegraeff & Bolch 1992; Hallegraeff, Steffensen & Wetherbee 1988). A comparative population genetic study of PST producing *Alexandrium* isolates from Japanese coastal waters and Australian coastal waters would enable this hypothesis to be investigated. So far, the scientist do not have a clear idea on how different *Alexandrium* species are linked or how the evolution happened in between the species along different coastal areas of the world. Single nucleotide level study could help to dig out these answers easily. This knowledge can also be used to develop population genetic study in other dinoflagellates in future. Evolution of different dinoflagellate species can also be well understood in future.

These suggested studies could create new opportunities to understand the PST producing *Alexandrium* species to establish early warning systems to protect the growing aquaculture shellfish industries. The knowledge will also contribute to develop future bloom mitigation plans. The unique technology that was successfully used for the first time in algae during this study period like Nanostring technology will lead to more research on other dinoflagellates or any other algae and help to understand if there is any harmful or beneficial activity. Developing the method for population genetic study using single nucleotide polymorphism (SNP) for an organism which does not have any reference genome is another success of this study. This knowledge will have a big impact on future population studies in algae and/or organisms which do not have any reference sequences available. This work will be considered a milestone in the evolution of algal studies in future.

Bibliography

Ajani, P., Brett, S., Krogh, M., Scanes, P., Webster, G., Armand, L. & assessment 2013, 'The risk of harmful algal blooms (HABs) in the oyster-growing estuaries of New South Wales, Australia', *Environmental Monitoring Assessment*, vol. 185, no. 6, pp. 5295-316.

Ajani, P., Hallegraeff, G. & Pritchard, T. 2001, 'Historic overview of algal blooms in marine and estuarine waters of New South Wales, Australia', vol. 123, Linnean Society of New South Wales, pp. 1-22.

Ajani, P., Harwood, D.T. & Murray, S.A. 2017, 'Recent trends in marine phycotoxins from Australian coastal waters', *Marine drugs*, vol. 15, no. 2, p. 33.

Akbar, M.A., Ahmad, A., Usup, G. & Bunawan, H. 2018, 'RNA-Seq as an emerging tool for marine dinoflagellate transcriptome analysis: process and challenges', *Processes*, vol. 6, no. 1, p. 5.

Allen, J.R., Roberts, T.M., Loeblich III, A.R. & Klotz, L.C. 1975, 'Characterization of the DNA from the dinoflagellate Crypthecodinium cohnii and implications for nuclear organization', *Cell*, vol. 6, no. 2, pp. 161-9.

Alpermann, T.J., Beszteri, B., John, U., Tillmann, U. & Cembella, A.D. 2009, 'Implications of life-history transitions on the population genetic structure of the toxigenic marine dinoflagellate *Alexandrium tamarense*', *Molecular Ecology*, vol. 18, no. 10, pp. 2122-33.

Alpermann, T.J., Tillmann, U., Beszteri, B., Cembella, A.D. & John, U. 2010, 'Phenotypic variation and genotypic diversity in a planktonic population of the toxigenic marine dinoflagellate Alexandrium tamarense (Dinophyceae) 1', *Journal of Phycology*, vol. 46, no. 1, pp. 18-32.

Amaya, O., Quintanilla, R., Stacy, B.A., Dechraoui Bottein, M., Flewelling, L.J., Hardy, R., Dueñas, C. & Ruiz, G. 2018, 'Large-scale sea turtle mortality events in El Salvador attributed to paralytic shellfish toxin-producing algae blooms', *Frontiers in Marine Science*, vol. 5, p. 411.

Anderson, D.M. 1997, 'Bloom dynamics of toxic *Alexandrium* species in the northeastern US. ', *Limnology and Oceanography*, vol. 42, no. 5part2, pp. 1009-22.

Anderson, D.M. 1998, 'Physiology and bloom dynamics of toxic *Alexandrium* species, with emphasis on life cycle transitions', *Nato Asi Series G Ecological Sciences*, vol. 41, pp. 29-48.

Anderson, D.M., Burkholder, J.M., Cochlan, W.P., Glibert, P.M., Gobler, C.J., Heil, C.A., Kudela, R.M., Parsons, M.L., Rensel, J.J. & Townsend, D.W. 2008, 'Harmful algal blooms and eutrophication: examining linkages from selected coastal regions of the United States', *Harmful algae*, vol. 8, no. 1, pp. 39-53.

Anderson, D.M., Cembella, A.D. & Hallegraeff, G.M. 2012, 'Progress in understanding harmful algal blooms: paradigm shifts and new technologies for research, monitoring, and management', *Annual review of marine science*, vol. 4, pp. 143-76.

Anderson, D.M., Glibert, P.M. & Burkholder, J.M. 2002, 'Harmful algal blooms and eutrophication: nutrient sources, composition, and consequences', *Estuaries*, vol. 25, no. 4, pp. 704-26.

Anderson, D.M., Hoagland, P., Kaoru, Y. & White, A.W. 2000, *Estimated annual economic impacts from harmful algal blooms (HABs) in the United States*, National Oceanic and Atmospheric Administration Norman OK National Severe ….

Anderson, D.M., Kulis, D.M., Sullivan, J.J., Hall, S. & Lee, C. 1990, 'Dynamics and physiology of saxitoxin production by the dinoflagellates *Alexandrium spp*', *Marine biology*, vol. 104, no. 3, pp. 511-24.

Anderson, P.D. 2012, 'Bioterrorism: toxins as weapons', *Journal of pharmacy practice*, vol. 25, no. 2, pp. 121-9.

Anderson, R.A. & Kawachi, M. 2005, 'Traditional microalgae isolation techniques. Algal culturing techniques. Edited by: Andersen RA. ', Series Traditional microalgae isolation techniques. Algal culturing techniques. Edited by: Andersen RA. Elsevier Inc, New York.

Asakawa, M., Gomez-Delan, G., Barte-Quilantang, M. & Ito, K. 2015, 'Paralytic shellfish poison (PSP)–producing dinoflagellate and PSP-infested organisms', *Marine Protists*, Springer, pp. 567-96.

Bachvaroff, T.R. & Place, A.R. 2008, 'From stop to start: tandem gene arrangement, copy number and trans-splicing sites in the dinoflagellate *Amphidinium carterae*', *PLoS One*, vol. 3, no. 8, p. e2929.

Backer, L.C., Fleming, L.E., Rowan, A., Cheng, Y., Benson, J., Pierce, R.H., Zaias, J., Bean, J., Bossart, G.D. & Johnson, D. 2003, 'Recreational exposure to aerosolized brevetoxins during Florida red tide events', *Harmful Algae*, vol. 2, no. 1, pp. 19-28.

Bagøien, E., Miranda, A., Reguera, B. & Franco, J.M. 1996, 'Effect of the toxic dinoflagellate Alexandrium minutum on the copepod Euterpina acutifrons'.

Bairoch, A. & Apweiler, R. 1996, 'The SWISS-PROT protein sequence data bank and its new supplement TREMBL', *Nucleic acids research*, vol. 24, no. 1, pp. 21-5.

Balech, E. 1985, 'The genus Alexandrium or Gonyaulax of the tamarensis group', *Toxic dinoflagellates*, pp. 33-8.

Balech, E. 1990, 'Four new dinoflagellates', *Helgoländer Meeresuntersuchungen*, vol. 44, no. 3, pp. 387-96.

Balech, E. 1995, *The Genus Alexandrium Halim (Dinoflagellata)*, vol. 452, Sherkin Island marine station, County Cork, Ireland.

Band-Schmidt, C.J., Bustillos-Guzmán, J.J., López-Cortés, D.J., Gárate-Lizárraga, I., Núñez-Vázquez, E.J. & Hernández-Sandoval, F.E. 2010, 'Ecological and physiological studies of *Gymnodinium catenatum* in the Mexican Pacific: A review', *Marine drugs*, vol. 8, no. 6, pp. 1935-61.

Band-Schmidt, C.J., Durán-Riveroll, L.M., Bustillos-Guzmán, J.J., Leyva-Valencia, I., López-Cortés, D.J., Núñez-Vázquez, E.J., Hernández-Sandoval, F.E. & Ramírez-Rodríguez, D.V. 2019, 'Paralytic toxin producing dinoflagellates in Latin America: Ecology and physiology', *Frontiers in Marine Science*, vol. 6, p. 42.

Banguera-Hinestroza, E., Eikrem, W., Mansour, H., Solberg, I., Curdia, J., Holtermann, K., Edvardsen, B. & Kaartvedt, S. 2016, 'Seasonality and toxin production of *Pyrodinium bahamense* in a Red Sea lagoon', *Harmful algae*, vol. 55, pp. 163-71.

Banks, S.C., Piggott, M.P., Williamson, J.E., Bové, U., Holbrook, N.J. & Beheregaray, L.B. 2007, 'Oceanic variability and coastal topography shape genetic structure in a long-dispersing sea urchin', *Ecology*, vol. 88, no. 12, pp. 3055-64.

Barton, S., Jenkins, J., Buckling, A., Schaum, C.E., Smirnoff, N., Raven, J.A. & Yvon-Durocher, G. 2020, 'Evolutionary temperature compensation of carbon fixation in marine phytoplankton', *Ecology letters*, vol. 23, no. 4, pp. 722-33.

Barua, A., Ajani, P.A., Ruvindy, R., Farrell, H., Zammit, A., Brett, S., Hill, D., Sarowar, C., Hoppenrath, M. & Murray, S.A. 2020, 'First detection of Paralytic Shellfish Toxins from *Alexandrium pacificum* above the regulatory limit in Blue Mussels (*Mytilus galloprovincialis*) in New South Wales, Australia', *Microorganisms*, vol. 8, no. 6, p. 905.

Beauchemin, M., Roy, S., Daoust, P., Dagenais-Bellefeuille, S., Bertomeu, T., Letourneau, L., Lang, B.F. & Morse, D. 2012, 'Dinoflagellate tandem array gene transcripts are highly conserved and not polycistronic', *Proceedings of the National Academy of Sciences*, vol. 109, no. 39, pp. 15793-8.

Bechard, A. 2019, 'Red tide at morning, tourists take warning? County-level economic effects of HABS on tourism dependent sectors', *Harmful algae*, vol. 85, p. 101689.

Berge, T., Hansen, P.J. & Moestrup, Ø. 2008, 'Prey size spectrum and bioenergetics of the mixotrophic dinoflagellate *Karlodinium armiger*', *Aquatic microbial ecology*, vol. 50, no. 3, pp. 289-99.

Bergkvist, J., Selander, E. & Pavia, H. 2008, 'Induction of toxin production in dinoflagellates: the grazer makes a difference', *Oecologia*, vol. 156, no. 1, pp. 147-54.

Blackburn, S.I., Bolch, C.J.S., Haskard, K.A. & Hallegraeff, G.M. 2001, 'Reproductive compatibility among four global populations of the toxic dinoflagellate *Gymnodinium catenatum* (Dinophyceae)', *Phycologia*, vol. 40, no. 1, pp. 78-87.

Blossom, H.E. & Hansen, P.J. 2020, 'The loss of mixotrophy in Alexandrium pseudogonyaulax: Implications for trade-offs between toxicity, mucus trap production, and phagotrophy', *Limnology and Oceanography*.

Bolch, C., Harwood, T., Untari, L., Murray, S., Hallegraeff, G. & Turnbull, A. 2014, '*Alexandrium tamarense* Group I as the cause of PST on the east coast of Tasmania, Australia', p. 29.

Bolch, C.J.S. & de Salas, M.F. 2007, 'A review of the molecular evidence for ballast water introduction of the toxic dinoflagellates *Gymnodinium catenatum* and the *Alexandrium "tamarensis complex"* to Australasia', *Harmful Algae*, vol. 6, no. 4, pp. 465-85.

Bolger, A.M., Lohse, M. & Usadel, B. 2014, 'Trimmomatic: a flexible trimmer for Illumina sequence data', *Bioinformatics*, vol. 30, no. 15, pp. 2114-20.

Bolyen, E., Rideout, J.R., Dillon, M.R., Bokulich, N.A., Abnet, C., Al-Ghalith, G.A., Alexander, H., Alm, E.J., Arumugam, M. & Asnicar, F. 2018, *QIIME 2: Reproducible, interactive, scalable, and extensible microbiome data science*, 2167-9843, PeerJ Preprints.

Boundy, M.J., Selwood, A.I., Harwood, D.T., McNabb, P.S. & Turner, A.D. 2015, 'Development of a sensitive and selective liquid chromatography–mass spectrometry method for high throughput analysis of paralytic shellfish toxins using graphitised carbon solid phase extraction', *Journal of Chromatography A*, vol. 1387, pp. 1-12.

Branco, S., Oliveira, M.M.M., Salgueiro, F., Vilar, M.C.P., Azevedo, S.M.F.O. & Menezes, M. 2020, 'Morphology and molecular phylogeny of a new PST-producing dinoflagellate species: *Alexandrium fragae sp. nov.*(Gonyaulacales, dinophyceae)', *Harmful Algae*, vol. 95, p. 101793.

Brandenburg, K.M., Velthuis, M. & Van de Waal, D.B. 2019, 'Meta-analysis reveals enhanced growth of marine harmful algae from temperate regions with warming and elevated CO2 levels', *Global change biology*, vol. 25, no. 8, pp. 2607-18.

Brosnahan, M.L., Fischer, A.D., Lopez, C.B., Moore, S.K. & Anderson, D.M. 2020, 'Cyst-forming dinoflagellates in a warming climate', *Harmful Algae*, vol. 91, p. 101728.

Bushnell, B. 2014, *BBMap: a fast, accurate, splice-aware aligner*, Lawrence Berkeley National Lab.(LBNL), Berkeley, CA (United States).

Campbell, A., McLeod, C., Pointon, A., Hudson, D. & Nicholls, C. 2013, *The 2012–13 Paralytic Shellfish Toxin Event in Tasmania Associated with Dinoflagellate Alga, Alexandrium tamarense*, vol. 93.

Cannon, J.A. 1990, 'Development and dispersal of red tides in the Port River, South Australia'.

Caruana, A.M.N., Le Gac, M., Hervé, F., Rovillon, G.A., Geffroy, S., Malo, F., Abadie, E. & Amzil, Z. 2020, '*Alexandrium pacificum* and *Alexandrium minutum*: Harmful or environmentally friendly?', *Marine Environmental Research*, p. 105014.

Casabianca, S., Cornetti, L., Capellacci, S., Vernesi, C. & Penna, A. 2017, 'Genome complexity of harmful microalgae', *Harmful algae*, vol. 63, pp. 7-12.

Casabianca, S., Penna, A., Pecchioli, E., Jordi, A., Basterretxea, G. & Vernesi, C. 2012, 'Population genetic structure and connectivity of the harmful dinoflagellate *Alexandrium minutum* in the Mediterranean Sea', *Proceedings of the Royal Society B: Biological Sciences*, vol. 279, no. 1726, pp. 129-38.

Catterall, W.A. 1985, 'The voltage sensitive sodium channel: a receptor for multiple neurotoxins', Series The voltage sensitive sodium channel: a receptor for multiple neurotoxins Elsevier Science Publishing Co., Inc.: New York, NY, USA, Toxic Dinoflagellates, pp. 329-42.

Cermeño, P. & Falkowski, P. 2009, 'Controls on diatom biogeography in the ocean', *Science*, vol. 325, no. 5947, pp. 1539-41.

Cestèle, S. & Catterall, W.A. 2000, 'Molecular mechanisms of neurotoxin action on voltage-gated sodium channels', *Biochimie*, vol. 82, no. 9-10, pp. 883-92.

Chang, F.H., Anderson, D.M., Kulis, D.M. & Till, D.G. 1997, 'Toxin production of *Alexandrium minutum* (Dinophyceae) from the bay of Plenty, New Zealand', *Toxicon*, vol. 35, no. 3, pp. 393-409.

Cho, Y., Tsuchiya, S., Omura, T., Koike, K., Oikawa, H., Konoki, K., Oshima, Y. & Yotsu-Yamashita, M. 2019, 'Metabolomic study of saxitoxin analogues and biosynthetic intermediates in dinoflagellates using 15 N-labelled sodium nitrate as a nitrogen source', *Scientific reports*, vol. 9, no. 1, pp. 1-11.

Chomérat, N., iti Gatti, C.M., Nézan, É. & Chinain, M. 2017, 'Studies on the benthic genus *Sinophysis* (Dinophysales, Dinophyceae) II. *S. canaliculata* from Rapa Island (French Polynesia)', *Phycologia*, vol. 56, no. 2, pp. 193-203.

Ciminiello, P., Fattorusso, E., Forino, M. & Montresor, M. 2000, 'Saxitoxin and neosaxitoxin as toxic principles of *Alexandrium andersoni* (Dinophyceae) from the Gulf of Naples, Italy', *Toxicon*, vol. 38, no. 12, pp. 1871-7.

Cirés, S., Delgado, A., González-Pleiter, M. & Quesada, A. 2017, 'Temperature Influences the Production and Transport of Saxitoxin and the Expression of sxt Genes in the Cyanobacterium *Aphanizomenon gracile*', *Toxins*, vol. 9, no. 10, p. 322.

Clément, A., Lincoqueo, L., Saldivia, M., Brito, C.G., Muñoz, F., Fernández, C., Pérez, F., Maluje, C.P., Martins, N.C.S. & Moncada, V. 2016, 'Climatic anomalies and harmful flagellates blooms in Southern Chile', pp. 34-7.

Coleman, M.A., Chambers, J., Knott, N.A., Malcolm, H.A., Harasti, D., Jordan, A. & Kelaher, B. 2011, 'Connectivity within and among a network of temperate marine reserves', *PloS one*, vol. 6, no. 5, p. e20168.

Coleman, M.A., Feng, M., Roughan, M., Cetina-Heredia, P. & Connell, S.D. 2013, 'Temperate shelf water dispersal by Australian boundary currents: implications for population connectivity', *Limnology and Oceanography: Fluids and Environments*, vol. 3, no. 1, pp. 295-309.

Coleman, M.A., Roughan, M., Macdonald, H.S., Connell, S.D., Gillanders, B.M., Kelaher, B.P. & Steinberg, P.D. 2011, 'Variation in the strength of continental boundary currents determines continent-wide connectivity in kelp', *Journal of Ecology*, vol. 99, no. 4, pp. 1026-32.

Condie, S.A., Oliver, E.C.J. & Hallegraeff, G.M. 2019, 'Environmental drivers of unprecedented *Alexandrium catenella* dinoflagellate blooms off eastern Tasmania, 2012–2018', *Harmful algae*, vol. 87, p. 101628.

Cusick, K.D. & Widder, E.A. 2014, 'Intensity differences in bioluminescent dinoflagellates impact foraging efficiency in a nocturnal predator', *Bulletin of Marine Science*, vol. 90, no. 3, pp. 797-811.

Cusick, K.D. & Widder, E.A.J.H.A. 2020, 'Bioluminescence and toxicity as driving factors in harmful algal blooms: Ecological functions and genetic variability', vol. 98, p. 101850.

Davies, W., Jakobsen, K.S. & Nordby, Ø. 1988, 'Characterization of DNA from the Dinoflagellate *Woloszynskia bostoniensis* ', *Journal of protozoology*, vol. 35, no. 3, pp. 418-22.

de Salas, M.F., van Emmerik, M.J., Hallegraeff, G.M., Negri, A.P., Vaillancourt, R.E. & Bolch, C.J. 2001, 'Toxic Australian *Alexandrium* dinoflagellates: introduced or indigenous', *Harmful algal blooms*, pp. 214-7.

Deeds, J.R., Landsberg, J.H., Etheridge, S.M., Pitcher, G.C. & Longan, S.W. 2008, 'Non-traditional vectors for paralytic shellfish poisoning', *Marine drugs*, vol. 6, no. 2, pp. 308-48.

Dia, A., Guillou, L., Mauger, S., Bigeard, E., Marie, D., Valero, M. & Destombe, C. 2014, 'Spatiotemporal changes in the genetic diversity of harmful algal blooms caused by the toxic dinoflagellate A lexandrium minutum', *Molecular ecology*, vol. 23, no. 3, pp. 549-60.

Dias, J., Muñoz, J., Huisman, J. & McDonald, J. 2015, 'Biosecurity monitoring of Harmful Algal Bloom (HAB) species in Western Australian waters: first confirmed record of *Alexandrium catenella* (Dinophyceae)', *BioInvasions Records*, vol. 4, no. 4, pp. 233-41.

Dobin, A., Davis, C.A., Schlesinger, F., Drenkow, J., Zaleski, C., Jha, S., Batut, P., Chaisson, M. & Gingeras, T.R. 2013, 'STAR: ultrafast universal RNA-seq aligner', *Bioinformatics*, vol. 29, no. 1, pp. 15-21.

Erdner, D.L., Richlen, M., McCauley, L.A.R. & Anderson, D.M. 2011, 'Diversity and dynamics of a widespread bloom of the toxic dinoflagellate *Alexandrium fundyense*', *PloS one*, vol. 6, no. 7, p. e22965.

Estrada, M., Sanchez, F.J. & Fraga, S. 1984, '*Gymnodinium catenatum* (Graham) en las rias gallegas (NO de Espana)'.

Farrell, H., Ajani, P., Brett, S., Zammit, A. & Murray, S. 2014, '*Alexandrium* species in New South Wales (NSW) coastal waters: historical distributions and identification of high-risk zones', Organising Committee, International Conference on Molluscan Shellfish Society, pp. 107-10.

Farrell, H., Brett, S., Ajani, P. & Murray, S. 2013, 'Distribution of the genus *Alexandrium* (Halim) and paralytic shellfish toxins along the coastline of New South Wales, Australia', *Marine pollution bulletin*, vol. 72, no. 1, pp. 133-45.

Farrell, H., Brett, S., Webster, G., Baker, P. & Zammit, A. 2014, 'A summary of harmful algal bloom monitoring and risk assessment within shellfish harvest areas in New South Wales, Australia', *Marine Fresh Water Harmful Algae*, p. 254.

Finlay, B.J. 2002, 'Global dispersal of free-living microbial eukaryote species', *Science*, vol. 296, no. 5570, pp. 1061-3.

Fischer, M.C., Rellstab, C., Leuzinger, M., Roumet, M., Gugerli, F., Shimizu, K.K., Holderegger, R. & Widmer, A. 2017, 'Estimating genomic diversity and population differentiation–an empirical comparison of microsatellite and SNP variation in *Arabidopsis halleri*', *BMC genomics*, vol. 18, no. 1, pp. 1-15.

Flynn, K.J., Mitra, A., Anestis, K., Anschütz, A.A., Calbet, A., Ferreira, G.D., Gypens, N., Hansen, P.J., John, U. & Martin, J.L. 2019, 'Mixotrophic protists and a new paradigm for marine ecology: where does plankton research go now?', *Journal of Plankton Research*, vol. 41, no. 4, pp. 375-91.

Foissner, W. 2007, 'Protist diversity and distribution: some basic considerations', *Protist diversity and geographical distribution*, Springer, pp. 1-8.

Frusher, S.D., Hobday, A.J., Jennings, S.M., Creighton, C., D'Silva, D., Haward, M., Holbrook, N.J., Nursey-Bray, M., Pecl, G.T. & van Putten, E.I. 2014, 'The short history of research in a marine climate change hotspot: from anecdote to adaptation in south-east Australia', *Reviews in Fish Biology and Fisheries*, vol. 24, no. 2, pp. 593-611.

Furio, E.F., Azanza, R.V., Fukuyo, Y. & Matsuoka, K. 2012, 'Review of geographical distribution of dinoflagellate cysts in Southeast Asian coasts', *Coastal marine science*, vol. 35, no. 1, pp. 20-33.

Galluzzi, L., Cegna, A., Casabianca, S., Penna, A., Saunders, N. & Magnani, M. 2011, 'Development of an oligonucleotide microarray for the detection and monitoring of marine dinoflagellates', *Journal of microbiological methods*, vol. 84, no. 2, pp. 234-42.

Gao, Y., Sassenhagen, I., Richlen, M.L., Anderson, D.M., Martin, J.L. & Erdner, D.L. 2019, 'Spatiotemporal genetic structure of regional-scale *Alexandrium catenella* dinoflagellate blooms explained by extensive dispersal and environmental selection', *Harmful algae*, vol. 86, pp. 46-54.

Garrison, E. & Marth, G. 2012, 'FreeBayes. arXiv preprint1207. 3907 [q-bio. GN][Internet', Series FreeBayes. arXiv preprint1207. 3907 [q-bio. GN][Internet.

Geiss, G.K., Bumgarner, R.E., Birditt, B., Dahl, T., Dowidar, N., Dunaway, D.L., Fell, H.P., Ferree, S., George, R.D. & Grogan, T. 2008, 'Direct multiplexed measurement of gene expression with color-coded probe pairs', *Nature biotechnology*, vol. 26, no. 3, pp. 317-25.

Genovesi, B., Berrebi, P., Nagai, S., Reynaud, N., Wang, J. & Masseret, E. 2015, 'Geographic structure evidenced in the toxic dinoflagellate *Alexandrium pacificum* Litaker (A. catenella–group IV (Whedon & Kofoid) Balech) along Japanese and Chinese coastal waters', *Marine pollution bulletin*, vol. 98, no. 1-2, pp. 95-105.

Glibert, P.M., Heil, C.A., Wilkerson, F.P. & Dugdale, R.C. 2018, 'Nutrients and harmful algal blooms: dynamic kinetics and flexible nutrition', *Global ecology and oceanography of harmful algal blooms*, Springer, pp. 93-112.

Gobler, C.J. 2020, 'Climate change and harmful algal blooms: insights and perspective', *Harmful Algae*, vol. 91, p. 101731.

Gobler, C.J., Doherty, O.M., Hattenrath-Lehmann, T.K., Griffith, A.W., Kang, Y. & Litaker, R.W. 2017, 'Ocean warming since 1982 has expanded the niche of toxic algal blooms in the North Atlantic and North Pacific oceans', *Proceedings of the National Academy of Sciences*, vol. 114, no. 19, pp. 4975-80.

Godhe, A. & Harntrom, K. 2010, 'Linking the planktonic and benthic habitat: genetic structure of the marine diatom *Skeletonema marinoi*', *Molecular Ecology*, vol. 19, no. 20, pp. 4478-90.

Godhe, A., Sjöqvist, C., Sildever, S., Sefbom, J., Harðardóttir, S., Bertos-Fortis, M., Bunse, C., Gross, S., Johansson, E. & Jonsson, P.R. 2016, 'Physical barriers and environmental gradients cause spatial and temporal genetic differentiation of an extensive algal bloom', *Journal of Biogeography*, vol. 43, no. 6, pp. 1130-42.

Grabherr, M.G., Haas, B.J., Yassour, M., Levin, J.Z., Thompson, D.A., Amit, I., Adiconis, X., Fan, L., Raychowdhury, R. & Zeng, Q. 2011, 'Trinity: reconstructing a full-length transcriptome without a genome from RNA-Seq data', *Nature biotechnology*, vol. 29, no. 7, p. 644.

Graham, H.W. 1943, '*Gymnodinium catenatum*, a new dinoflagellate from the Gulf of California', *Transactions of the American Microscopical Society*, vol. 62, no. 3, pp. 259-61.

Griffith, A.W. & Gobler, C.J. 2020, 'Harmful algal blooms: a climate change co-stressor in marine and freshwater ecosystems', *Harmful Algae*, vol. 91, p. 101590.

Grzebyk, D., Béchemin, C., Ward, C.J., Vérité, C., Codd, G.A. & Maestrini, S.Y. 2003, 'Effects of salinity and two coastal waters on the growth and toxin content of the dinoflagellate *Alexandrium minutum*', *Journal of Plankton Research*, vol. 25, no. 10, pp. 1185-99.

Guindon, S., Dufayard, J.F., Lefort, V., Anisimova, M., Hordijk, W. & Gascuel, O. 2010, 'New algorithms and methods to estimate maximum-likelihood phylogenies: assessing the performance of PhyML 3.0', *Systematic biology*, vol. 59, no. 3, pp. 307-21.

Hackett, J.D., Anderson, D.M., Erdner, D.L. & Bhattacharya, D. 2004, 'Dinoflagellates: a remarkable evolutionary experiment', *American Journal of Botany*, vol. 91, no. 10, pp. 1523-34.

Hackett, J.D., Wisecaver, J.H., Brosnahan, M.L., Kulis, D.M., Anderson, D.M., Bhattacharya, D., Plumley, F.G., Erdner, D.L. & evolution 2013, 'Evolution of saxitoxin synthesis in cyanobacteria and dinoflagellates', *Molecular biology*, vol. 30, no. 1, pp. 70-8.

Hadjadji, I., Laabir, M., Frihi, H., Collos, Y., Shao, Z.J., Berrebi, P., Abadie, E., Amzil, Z., Chomérat, N. & Rolland, J.L. 2020, 'Unsuspected intraspecific variability in the toxin production, growth and morphology of the dinoflagellate *Alexandrium pacificum* Litaker sp. nov (Group IV) blooming in a South Western Mediterranean marine ecosystem, Annaba Bay (Algeria)', *Toxicon*.

Halim, Y. 1960, '*Alexandrium minutum* nov. g. nov. sp. dinoflagellé provocant des «eaux rouges»', *Vie et milieu*, vol. 11, no. 1, pp. 102-5.

Hallegraeff, G.M. 1992, 'Harmful algal blooms in the Australian region', *Marine pollution bulletin*, vol. 25, no. 5-8, pp. 186-90.

Hallegraeff, G.M. 1993, 'A review of harmful algal blooms and their apparent global increase', *Phycologia*, vol. 32, no. 2, pp. 79-99.

Hallegraeff, G.M., Blackburn, S.I., Doblin, M.A. & Bolch, C.J.S. 2012, 'Global toxicology, ecophysiology and population relationships of the chainforming PST dinoflagellate *Gymnodinium catenatum*', *Harmful Algae*, vol. 14, pp. 130-43.

Hallegraeff, G.M. & Bolch, C.J. 1992, 'Transport of diatom and dinoflagellate resting spores in ships' ballast water: implications for plankton biogeography and aquaculture', *Journal of plankton research*, vol. 14, no. 8, pp. 1067-84.

Hallegraeff, G.M., Bolch, C.J.S., Blackburn, S.I. & Oshima, Y. 1991, 'Species of the toxigenic dinoflagellate genus *Alexandrium* in southeastern Australian waters', *Botanica marina*, vol. 34, no. 6, pp. 575-88.

Hallegraeff, G.M., Bolch, C.J.S., Hill, D.R.A., Jameson, I., LeRoi, J.M., McMinn, A., Murray, S., de Salas, M.F. & Saunders, K. 2010, *Algae of Australia: phytoplankton of temperate coastal waters*.

Hallegraeff, G.M., McCausland, M.A. & Brown, R.K. 1995, 'Early warning of toxic dinoflagellate blooms of *Gymnodinium catenatum* in southern Tasmanian waters', *Journal of plankton research*, vol. 17, no. 6, pp. 1163-76.

Hallegraeff, G.M. & Reid, D.D. 1986, 'Phytoplankton species successions and their hydrological environment at a coastal station off Sydney', *Marine and Freshwater Research*, vol. 37, no. 3, pp. 361-77.

Hallegraeff, G.M., Schweibold, L., Jaffrezic, E., Rhodes, L., MacKenzie, L., Hay, B. & Farrell, H. 2020, 'Overview of Australian and New Zealand harmful algal species occurrences and their societal impacts in the period 1985 to 2018, including a compilation of historic records', *Harmful Algae*, p. 101848.

Hallegraeff, G.M., Steffensen, D.A. & Wetherbee, R.J. 1988, 'Three estuarine Australian dinoflagellates that can produce paralytic shellfish toxins', *Journal of plankton research*, vol. 10, no. 3, pp. 533-41.

Hansen, G., Daugbjerg, N. & Franco, J.M. 2003, 'Morphology, toxin composition and LSU rDNA phylogeny of *Alexandrium minutum* (Dinophyceae) from Denmark, with some morphological observations on other European strains', *Harmful Algae*, vol. 2, no. 4, pp. 317-35.

Harwood, D.T., Boundy, M., Selwood, A.I., van Ginkel, R., MacKenzie, L. & McNabb, P.S. 2013, 'Refinement and implementation of the Lawrence method (AOAC 2005.06) in a commercial laboratory: assay performance during an *Alexandrium catenella* bloom event', *Harmful Algae*, vol. 24, pp. 20-31.

Heisler, J., Glibert, P.M., Burkholder, J.M., Anderson, D.M., Cochlan, W., Dennison, W.C., Dortch, Q., Gobler, C.J., Heil, C.A. & Humphries, E. 2008, 'Eutrophication and harmful algal blooms: a scientific consensus', *Harmful algae*, vol. 8, no. 1, pp. 3-13.

Hennon, G.M.M. & Dyhrman, S.T. 2020, 'Progress and promise of omics for predicting the impacts of climate change on harmful algal blooms', *Harmful algae*, vol. 91, p. 101587.

Hong, J., Talapatra, S., Katz, J., Tester, P.A., Waggett, R.J. & Place, A.R. 2012, 'Algal toxins alter copepod feeding behavior', *PloS one*, vol. 7, no. 5, p. e36845.

Hoppenrath, M., Elbrächter, M. & Drebes, G. 2009, 'Marine phytoplankton'.

Howe, C.J., Nisbet, R.E.R. & Barbrook, A.C. 2008, 'The remarkable chloroplast genome of dinoflagellates', *Journal of experimental botany*, vol. 59, no. 5, pp. 1035-45.

Irwin, N.A.T., Martin, B.J.E., Young, B.P., Browne, M.J.G., Flaus, A., Loewen, C.J.R., Keeling, P.J. & Howe, L.J. 2018, 'Viral proteins as a potential driver of histone depletion in dinoflagellates', *Nature communications*, vol. 9, no. 1, pp. 1-8.

Jaeckisch, N., Yang, I., Wohlrab, S., Glöckner, G., Kroymann, J., Vogel, H., Cembella, A. & John, U. 2011, 'Comparative genomic and transcriptomic characterization of the toxigenic marine dinoflagellate *Alexandrium ostenfeldii*', *PLoS One*, vol. 6, no. 12, p. e28012.

Jeong, H.J., Du Yoo, Y., Park, J.Y., Song, J.Y., Kim, S.T., Lee, S.H., Kim, K.Y. & Yih, W.H. 2005, 'Feeding by phototrophic red-tide dinoflagellates: five species newly revealed and six species previously known to be mixotrophic', *Aquatic microbial ecology*, vol. 40, no. 2, pp. 133-50.

John, E.H. & Flynn, K.J. 2002, 'Modelling changes in paralytic shellfish toxin content of dinoflagellates in response to nitrogen and phosphorus supply', *Marine Ecology Progress Series*, vol. 225, pp. 147-60.

John, U., Litaker, R.W., Montresor, M., Murray, S., Brosnahan, M.L. & Anderson, D.M. 2014, 'Formal revision of the *Alexandrium tamarense* species complex (Dinophyceae) taxonomy: the introduction of five species with emphasis on molecular-based (rDNA) classification', *Protist*, vol. 165, no. 6, pp. 779-804.

John, U., Tillmann, U., Hülskötter, J., Alpermann, T.J., Wohlrab, S. & Van de Waal, D.B. 2015, 'Intraspecific facilitation by allelochemical mediated grazing protection within a toxigenic dinoflagellate population', *Proceedings of the Royal Society B: Biological Sciences*, vol. 282, no. 1798, p. 20141268.

Johnson, J.G., Morey, J.S., Neely, M.G., Ryan, J.C. & Van Dolah, F.M. 2012, 'Transcriptome remodeling associated with chronological aging in the dinoflagellate, *Karenia brevis*', *Marine genomics*, vol. 5, pp. 15-25.

Jones, G.D., Williams, E.P., Place, A.R., Jagus, R. & Bachvaroff, T.R. 2015, 'The alveolate translation initiation factor 4E family reveals a custom toolkit for translational control in core dinoflagellates', *BMC evolutionary biology*, vol. 15, no. 1, pp. 1-12.

Juhl, A.R., Trainer, V.L. & Latz, M. 2001, 'Effect of fluid shear and irradiance on population growth and cellular toxin content of the dinoflagellate *Alexandrium fundyense*', *Limnology and Oceanography*, vol. 46, no. 4, pp. 758-64.

Kalaitzis, J.A., Chau, R., Kohli, G.S., Murray, S.A. & Neilan, B.A. 2010, 'Biosynthesis of toxic naturally-occurring seafood contaminants', *Toxicon*, vol. 56, no. 2, pp. 244-58.

Kantiani, L., Llorca, M., Sanchís, J., Farré, M. & Barceló, D. 2010, 'Emerging food contaminants: a review', *Analytical Bioanalytical Chemistry*, vol. 398, no. 6, pp. 2413-27.

Kao, C.Y. 1993, 'Paralytic shellfish poisoning', in I.R. Falconer (ed.), *Algal toxins in seafood and drinking water*, vol. 75, University of Adelaide, Adelaide, Australia, p. 86.

Kearse, M., Moir, R., Wilson, A., Stones-Havas, S., Cheung, M., Sturrock, S., Buxton, S., Cooper, A., Markowitz, S. & Duran, C. 2012, 'Geneious Basic: an integrated and extendable desktop software platform for the organization and analysis of sequence data', *Bioinformatics*, vol. 28, no. 12, pp. 1647-9.

Keller, M.D., Selvin, R.C., Claus, W. & Guillard, R.R.L. 1987, 'Media for the culture of oceanic ultraphytoplankton 1, 2', *Journal of phycology*, vol. 23, no. 4, pp. 633-8.

Kellmann, R., Mihali, T.K., Jeon, Y.J., Pickford, R., Pomati, F. & Neilan, B.A. 2008, 'Biosynthetic intermediate analysis and functional homology reveal a saxitoxin gene cluster in cyanobacteria', *Applied environmental microbiology*, vol. 74, no. 13, pp. 4044-53.

Kellmann, R., Stüken, A., Orr, R.J.S., Svendsen, H.M. & Jakobsen, K.S. 2010, 'Biosynthesis and molecular genetics of polyketides in marine dinoflagellates', *Marine drugs*, vol. 8, no. 4, pp. 1011-48.

Kim, D., Oda, T., Muramatsu, T., Kim, D., Matsuyama, Y. & Honjo, T. 2002, 'Possible factors responsible for the toxicity of *Cochlodinium polykrikoides,* a red tide phytoplankton', *Comparative Biochemistry Physiology Part C: Toxicology Pharmacology*, vol. 132, no. 4, pp. 415-23.

Koslow, J.A., Pesant, S., Feng, M., Pearce, A., Fearns, P., Moore, T., Matear, R. & Waite, A. 2008, 'The effect of the Leeuwin Current on phytoplankton biomass and production off Southwestern Australia', *Journal of Geophysical Research: Oceans*, vol. 113, no. C7.

Kremp, A., Hinners, J., Klais, R., Leppänen, A. & Kallio, A. 2018, 'Patterns of vertical cyst distribution and survival in 100-year-old sediment archives of three spring dinoflagellate species from the Northern Baltic Sea', *European Journal of Phycology*, vol. 53, no. 2, pp. 135-45.

Kremp, A., Oja, J., LeTortorec, A.H., Hakanen, P., Tahvanainen, P., Tuimala, J. & Suikkanen, S. 2016, 'Diverse seed banks favour adaptation of microalgal populations to future climate conditions', *Environmental Microbiology*, vol. 18, no. 2, pp. 679-91.

Krock, B., Seguel, C.G. & Cembella, A.D. 2007, 'Toxin profile of *Alexandrium catenella* from the Chilean coast as determined by liquid chromatography with fluorescence detection and liquid chromatography coupled with tandem mass spectrometry', *Harmful Algae*, vol. 6, no. 5, pp. 734-44.

Laabir, M., Amzil, Z., Lassus, P., Masseret, E., Tapilatu, Y., De Vargas, R. & Grzebyk, D. 2007, 'Viability, growth and toxicity of *Alexandrium catenella* and *Alexandrium minutum* (Dinophyceae) following ingestion and gut passage in the oyster *Crassostrea gigas*', *Aquatic Living Resources*, vol. 20, no. 1, pp. 51-7.

LaJeunesse, T.C., Lambert, G., Andersen, R.A., Coffroth, M.A. & Galbraith, D.W. 2005, 'Sybiodinium (Pyrrophyta) genome size (DNA content) are smallest among dinoflagellates', *Journal of Phycology*, vol. 41, no. 4, pp. 880-6.

Lakeman, M.B., von Dassow, P. & Cattolico, R.A. 2009, 'The strain concept in phytoplankton ecology', *Harmful Algae*, vol. 8, no. 5, pp. 746-58.

Landsberg, J.H. 2002, 'The effects of harmful algal blooms on aquatic organisms', *Reviews in Fisheries Science*, vol. 10, no. 2, pp. 113-390.

Landsberg, J.H., Hall, S., Johannessen, J.N., White, K.D., Conrad, S.M., Abbott, J.P., Flewelling, L.J., Richardson, R.W., Dickey, R.W. & Jester, E.L.E. 2006, 'Saxitoxin puffer fish poisoning in the United States, with the first report of *Pyrodinium bahamense* as the putative toxin source', *Environmental health perspectives*, vol. 114, no. 10, pp. 1502-7.

Larkin, M.A., Blackshields, G., Brown, N.P., Chenna, R., McGettigan, P.A., McWilliam, H., Valentin, F., Wallace, I.M., Wilm, A. & Lopez, R. 2007, 'Clustal W and Clustal X version 2.0', *Bioinformatics*, vol. 23, no. 21, pp. 2947-8.

Lawrence, J.F., Niedzwiadek, B. & Menard, C. 2005, 'Quantitative determination of paralytic shellfish poisoning toxins in shellfish using prechromatographic oxidation and liquid chromatography with fluorescence detection: collaborative study', *Journal of AOAC International*, vol. 88, no. 6, pp. 1714-32.

Le Gac, M., Metegnier, G., Chomérat, N., Malestroit, P., Quéré, J., Bouchez, O., Siano, R., Destombe, C., Guillou, L. & Chapelle, A. 2016, 'Evolutionary processes and cellular functions underlying divergence in Alexandrium minutum', *Molecular Ecology*, vol. 25, no. 20, pp. 5129-43.

Le Messurier, D.H. 1935, 'A Survey of Mussels on a Portion of the Australian Coast', *Medical Journal of Australia*, vol. 1, pp. 490-92.

Le, Q.H., Jovine, R., Markovic, P. & Morse, D. 2001, 'Peridinin-chlorophyll a-protein is not implicated in the photosynthesis rhythm of the dinoflagellate Gonyaulax despite circadian regulation of its translation', *Biological rhythm research*, vol. 32, no. 5, pp. 579-94.

Leaw, C.P., Lim, P.T., Ng, B.K., Cheah, M.Y., Ahmad, A. & Usup, G. 2005, 'Phylogenetic analysis of *Alexandrium* species and *Pyrodinium bahamense* (Dinophyceae) based on theca morphology and nuclear ribosomal gene sequence', *Phycologia*, vol. 44, no. 5, pp. 550-65.

Li, H., Handsaker, B., Wysoker, A., Fennell, T., Ruan, J., Homer, N., Marth, G., Abecasis, G. & Durbin, R. 2009, 'The sequence alignment/map format and SAMtools', *Bioinformatics*, vol. 25, no. 16, pp. 2078-9.

Lidie, K.B. & Van Dolah, F.M. 2007, 'Spliced leader RNA-mediated trans-splicing in a dinoflagellate, *Karenia brevis*', *Journal of Eukaryotic Microbiology*, vol. 54, no. 5, pp. 427-35.

Lilly, E.L., Halanych, K.M. & Anderson, D.M. 2007, 'Species boundaries and global biogeography of the Alexandrium tamarense complex (Dinophyceae) 1', *Journal of Phycology*, vol. 43, no. 6, pp. 1329-38.

Lim, P.T., Leaw, C.P., Usup, G., Kobiyama, A., Koike, K. & Ogata, T. 2006, 'Effects of time and temperature on growth, nitrate uptake, and toxin production of two tropical dinoflagellates: *Alexandrium tamiyavanichii* and *Alexandrium minutum* (Dinophyceae) 1', *Journal of Phycology*, vol. 42, no. 4, pp. 786-99.

Lim, P.T. & Ogata, T. 2005, 'Salinity effect on growth and toxin production of four tropical *Alexandrium* species (Dinophyceae)', *Toxicon*, vol. 45, no. 6, pp. 699-710.

Lin, S. 2011, 'Genomic understanding of dinoflagellates', *Research in microbiology*, vol. 162, no. 6, pp. 551-69.

Lin, S., Cheng, S., Song, B., Zhong, X., Lin, X., Li, W., Li, L., Zhang, Y., Zhang, H. & Ji, Z. 2015, 'The *Symbiodinium kawaguti*i genome illuminates dinoflagellate gene expression and coral symbiosis', *Science*, vol. 350, no. 6261, pp. 691-4.

Lin, S., Zhang, H., Zhuang, Y., Tran, B. & Gill, J. 2010, 'Spliced leader–based metatranscriptomic analyses lead to recognition of hidden genomic features in dinoflagellates', *Proceedings of the National Academy of Sciences*, vol. 107, no. 46, pp. 20033-8.

Liu, N., Chen, L., Wang, S., Oh, C. & Zhao, H. 2005, 'Comparison of single-nucleotide polymorphisms and microsatellites in inference of population structure', vol. 6, BioMed Central, pp. 1-5.

Llewellyn, L.E. 2006, 'Saxitoxin, a toxic marine natural product that targets a multitude of receptors', *Natural product reports*, vol. 23, no. 2, pp. 200-22.

Lundgreen, K. 2018, 'Methods to verify organism viability in treated ballast water', University of Southern Denmark.

Lynch, M.D.J. & Neufeld, J.D. 2015, 'Ecology and exploration of the rare biosphere', *Nature Reviews Microbiology*, vol. 13, no. 4, pp. 217-29.

Ma, H., Krock, B., Tillmann, U. & Cembella, A. 2009, 'Preliminary characterization of extracellular allelochemicals of the toxic marine dinoflagellate *Alexandrium tamarense* using a Rhodomonas salina bioassay', *Marine drugs*, vol. 7, no. 4, pp. 497-522.

MacKenzie, L., de Salas, M., Adamson, J. & Beuzenberg, V. 2004, 'The dinoflagellate genus *Alexandrium (Halim*) in New Zealand coastal waters: comparative morphology, toxicity and molecular genetics', *Harmful Algae*, vol. 3, no. 1, pp. 71-92.

Maclean, J.L. 1989, 'Indo-Pacific red tides, 1985–1988', *Marine Pollution Bulletin*, vol. 20, no. 7, pp. 304-10.

Masseret, E., Grzebyk, D., Nagai, S., Genovesi, B., Lasserre, B., Laabir, M., Collos, Y., Vaquer, A. & Berrebi, P. 2009, 'Unexpected genetic diversity among and within populations of the toxic dinoflagellate *Alexandrium catenella* as revealed by nuclear microsatellite markers', *Applied Environmental Microbiology*, vol. 75, no. 7, pp. 2037-45.

McCue, K.A. & Holtsford, T.P. 1998, 'Seed bank influences on genetic diversity in the rare annual *Clarkia springvillensis* (Onagraceae)', *American Journal of Botany*, vol. 85, no. 1, pp. 30-6.

McEwan, M., Humayun, R., Slamovits, C.H. & Keeling, P. 2008, 'Nuclear genome sequence survey of the dinoflagellate *Heterocapsa triquetra*', *Journal of Eukaryotic Microbiology*, vol. 55, no. 6, pp. 530-5.

McKenna, A., Hanna, M., Banks, E., Sivachenko, A., Cibulskis, K., Kernytsky, A., Garimella, K., Altshuler, D., Gabriel, S. & Daly, M. 2010, 'The Genome Analysis Toolkit: a MapReduce framework for analyzing next-generation DNA sequencing data', *Genome research*, vol. 20, no. 9, pp. 1297-303.

McMurdie, P.J. & Holmes, S. 2013, 'phyloseq: an R package for reproducible interactive analysis and graphics of microbiome census data', vol. 8, no. 4.

Medlin, L.K., Sáez, A.G. & Young, J.R. 2008, 'A molecular clock for coccolithophores and implications for selectivity of phytoplankton extinctions across the K/T boundary', *Marine micropaleontology*, vol. 67, no. 1-2, pp. 69-86.

Mihali, T.K., Kellmann, R. & Neilan, B.A. 2009, 'Characterisation of the paralytic shellfish toxin biosynthesis gene clusters in *Anabaena circinalis* AWQC131C and *Aphanizomenon* sp. NH-5', *BMC biochemistry*, vol. 10, no. 1, p. 8.

Mizushima, K. & Matsuoka, K. 2004, 'Vertical distribution and germination ability of *Alexandrium spp*. cysts (Dinophyceae) in the sediments collected from Kure Bay of the Seto Inland Sea, Japan', *Phycological Research*, vol. 52, no. 4, pp. 408-13.

Moore, S.K., Mantua, N.J. & Salathe Jr, E.P. 2011, 'Past trends and future scenarios for environmental conditions favoring the accumulation of paralytic shellfish toxins in Puget Sound shellfish', *Harmful Algae*, vol. 10, no. 5, pp. 521-9.

Morey-Gaines, G. 1982, '*Gymnodinium catenatum* Graham (Dinophyceae): morphology and affinities with armoured forms', *Phycologia*, vol. 21, no. 2, pp. 154-63.

Morey, J.S., Monroe, E.A., Kinney, A.L., Beal, M., Johnson, J.G., Hitchcock, G.L. & Van Dolah, F.M. 2011, 'Transcriptomic response of the red tide dinoflagellate, Karenia brevis, to nitrogen and phosphorus depletion and addition', *BMC genomics*, vol. 12, no. 1, p. 346.

Moustafa, A., Loram, J.E., Hackett, J.D., Anderson, D.M., Plumley, F.G. & Bhattacharya, D. 2009, 'Origin of saxitoxin biosynthetic genes in cyanobacteria', *PloS one*, vol. 4, no. 6, p. e5758.

Müller, M.N., Mardones, J.I. & Dorantes-Aranda, J. 2020, 'Harmful Algal Blooms (HABs) in Latin America', *Frontiers in Marine Science*, vol. 7, p. 34.

Murray, S., John, U. & Kremp, A. 2015, '*Alexandrium spp*: genetic and ecological factors influencing saxitoxin production and proliferation', *Climate Change Marine Freshwater Toxins*, pp. 123-39.

Murray, S.A., Diwan, R., Orr, R.J.S., Kohli, G.S. & John, U. 2015, 'Gene duplication, loss and selection in the evolution of saxitoxin biosynthesis in alveolates', *Molecular phylogenetics and evolution*

vol. 92, pp. 165-80.

Murray, S.A., Hoppenrath, M., Orr, R.J.S., Bolch, C.J., John, U., Diwan, R., Yauwenas, R., Harwood, T., de Salas, M.F. & Neilan, B. 2014, '*Alexandrium diversaporum* sp. nov., a new non-saxitoxin producing species: Phylogeny, morphology and sxtA genes', *Harmful algae*, vol. 31, pp. 54-65.

Murray, S.A., Suggett, D.J., Doblin, M.A., Kohli, G.S., Seymour, J.R., Fabris, M. & Ralph, P. 2016, 'Unravelling the functional genetics of dinoflagellates: a review of approaches and opportunities', *Perspect. Phycol*, vol. 3, no. 1, pp. 37-52.

Murray, S.A., Wiese, M., Neilan, B.A., Orr, R.J.S., de Salas, M., Brett, S. & Hallegraeff, G. 2012, 'A reinvestigation of saxitoxin production and sxtA in the 'non-toxic'*Alexandrium tamarense* Group V clade', *Harmful Algae*, vol. 18, pp. 96-104.

Murray, S.A., Wiese, M., Stüken, A., Brett, S., Kellmann, R., Hallegraeff, G. & Neilan, B.A. 2011, 'sxtA-based quantitative molecular assay to identify saxitoxin-producing harmful algal blooms in marine waters', *Applied and Environmental Microbiology*, vol. 77, no. 19, pp. 7050-7.

Nagai, S., Lian, C., Yamaguchi, S., Hamaguchi, M., Matsuyama, Y., Itakura, S., Shimada, H., Kaga, S., Yamauchi, H. & Sonda, Y.J.o.P. 2007, 'Microsatellite markers reveal population genetic structure of the toxic dinoflagellate *Alexandrium tamarense* (Dinophyceae) in Japanese coastal waters 1', *Journal of Phycology*

vol. 43, no. 1, pp. 43-54.

Nakamura, H., Kishi, Y., Shimomura, O., Morse, D. & Hastings, J.W. 1989, 'Structure of dinoflagellate luciferin and its enzymic and nonenzymic air-oxidation products', *Journal of the American Chemical Society*, vol. 111, no. 19, pp. 7607-11.

Narasimhan, V., Danecek, P., Scally, A., Xue, Y., Tyler-Smith, C. & Durbin, R. 2016, 'BCFtools/RoH: a hidden Markov model approach for detecting autozygosity from next-generation sequencing data', *Bioinformatics*, vol. 32, no. 11, pp. 1749-51.

Natsuike, M., Oikawa, H., Matsuno, K., Yamaguchi, A. & Imai, I. 2017, 'The physiological adaptations and toxin profiles of the toxic *Alexandrium fundyense* on the eastern Bering Sea and Chukchi Sea shelves', *Harmful Algae*, vol. 63, pp. 13-22.

Negri, A., Llewellyn, L., Doyle, J., Webster, N., Frampton, D. & Blackburn, S. 2003, 'Paralytic Shellfish toxins are restricted to few species among Australia's taxonomic diversity of cultured microalgae', *Journal of Phycology*, vol. 39, no. 4, pp. 663-7.

Negri, A.P., Bolch, C.J.S., Geier, S., Green, D.H., Park, T.G. & Blackburn, S.I. 2007, 'Widespread presence of hydrophobic paralytic shellfish toxins in *Gymnodinium catenatum*', *Harmful Algae*, vol. 6, no. 6, pp. 774-80.

Nguyen-Ngoc, L. 2004, 'An autecological study of the potentially toxic dinoflagellate *Alexandrium affine* isolated from Vietnamese waters', *Harmful Algae*, vol. 3, no. 2, pp. 117-29.

Ni, G., Strom, T.M., Pausch, H., Reimer, C., Preisinger, R., Simianer, H. & Erbe, M. 2015, 'Comparison among three variant callers and assessment of the accuracy of imputation from SNP array data to whole-genome sequence level in chicken', *BMC genomics*, vol. 16, no. 1, pp. 1-12.

Norris, R.D. 2000, 'Pelagic species diversity, biogeography, and evolution', *Paleobiology*, vol. 26, no. sp4, pp. 236-58.

NSWDPI. 2019, *Aquaculture production report 2018-2019,* https://www.dpi.nsw.gov.au/__data/assets/pdf_file/0015/1191021/Provisional-Production-Report-2018-2019.pdf.

NSWFA. 2015, *Marine biotoxin management plan,* https://www.foodauthority.nsw.gov.au/sites/default/files/_Documents/industry/marine_biotoxin_management_plan.pdf.

NSWFA. 2017, *Phytoplankton and biotoxins in NSW shellfish Aquaculture areas,* https://www.foodauthority.nsw.gov.au/sites/default/files/_Documents/scienceandtechnical/phytoplankton_and_biotoxin_risk_assessment.pdf.

Nunn, G.B., Theisen, B.F., Christensen, B. & Arctander, P. 1996, 'Simplicity-correlated size growth of the nuclear 28S ribosomal RNA D3 expansion segment in the crustacean order Isopoda', *Journal of Molecular Evolution*, vol. 42, no. 2, pp. 211-23.

Orlova, T.Y., Selina, M.S., Lilly, E.L., Kulis, D.M. & Anderson, D.M. 2007, 'Morphogenetic and toxin composition variability of *Alexandrium tamarense* (Dinophyceae) from the east coast of Russia', *Phycologia*, vol. 46, no. 5, pp. 534-48.

Orr, R.J.S., Stï, A., Murray, S.A. & Jakobsen, K.S. 2013a, 'Evolutionary acquisition and loss of saxitoxin biosynthesis in dinoflagellates: the second "core" gene, sxtG', *Applied environmental microbiology*, vol. 79, no. 7, pp. 2128-36.

Orr, R.J.S., Stï, A., Murray, S.A. & Jakobsen, K.S. 2013b, 'Evolutionary acquisition and loss of saxitoxin biosynthesis in dinoflagellates: the second "core" gene, sxtG', *Applied and Environmental Microbiology*, vol. 79, no. 7, pp. 2128-36.

Orr, R.J.S., Stüken, A., Rundberget, T., Eikrem, W. & Jakobsen, K.S. 2011, 'Improved phylogenetic resolution of toxic and non-toxic *Alexandrium* strains using a concatenated rDNA approach', *Harmful Algae*, vol. 10, no. 6, pp. 676-88.

Oshima, Y., Blackburn, S.I. & Hallegraeff, G.M. 1993, 'Comparative study on paralytic shellfish toxin profiles of the dinoflagellate *Gymnodinium catenatum* from three different countries', *Marine Biology*, vol. 116, no. 3, pp. 471-6.

Oshima, Y., Hasegawa, M., Yasumoto, T., Hallegraeff, G. & Blackburn, S. 1987, 'Dinoflagellate *Gymnodinium catenatum* as the source of paralytic shellfish toxins in Tasmanian shellfish', *Toxicon*, vol. 25, no. 10, pp. 1105-11.

Ouellette, M. & Papadopoulou, B. 2009, 'Coordinated gene expression by post-transcriptional regulons in African trypanosomes', *Journal of biology*, vol. 8, no. 11, p. 100.

Palumbi, S.R. 1992, 'Marine speciation on a small planet', *Trends in ecology & evolution*, vol. 7, no. 4, pp. 114-8.

Paradis, E., Claude, J. & Strimmer, K. 2004, 'APE: analyses of phylogenetics and evolution in R language', *Bioinformatics*, vol. 20, no. 2, pp. 289-90.

Paredes, J., Varela, D., Martínez, C., Zúñiga, A., Correa, K., Villarroel, A. & Olivares, B. 2019, 'Population genetic structure at the northern edge of the distribution of *Alexandrium catenella* in the Patagonian fjords and its expansion along the open Pacific Ocean coast', *Frontiers in Marine Science*, vol. 5, p. 532.

Perini, F., Galluzzi, L., Dell'Aversano, C., Iacovo, E.D., Tartaglione, L., Ricci, F., Forino, M., Ciminiello, P. & Penna, A. 2014, 'SxtA and sxtG gene expression and toxin production in the Mediterranean *Alexandrium minutum (*Dinophyceae)', *Marine drugs*, vol. 12, no. 10, pp. 5258-76.

Probert, I. 1999, 'Sexual reproduction and ecophysiology of the marine dinoflagellate *Alexandrium minutum* Halim', University of Westminster.

Reinfelder, J.R. 2011, 'Carbon concentrating mechanisms in eukaryotic marine phytoplankton', *Annual review of marine science*, vol. 3, pp. 291-315.

Rengefors, K. & Anderson, D.M. 1998, 'Environmental and endogenous regulation of cyst germination in two freshwater dinoflagellates', *Journal of Phycology*, vol. 34, no. 4, pp. 568-77.

Richlen, M.L., Erdner, D.L., McCauley, L.A.R., Libera, K. & Anderson, D.M. 2012, 'Extensive genetic diversity and rapid population differentiation during blooms of *Alexandrium fundyense* (D inophyceae) in an isolated salt pond on Cape Cod, MA, USA', *Ecology and Evolution*, vol. 2, no. 10, pp. 2588-99.

Ridgway, K. & Hill, K. 2012, 'East Australian Current. A marine climate change impacts and adaptation report card for Australia', Series East Australian Current. A marine climate change impacts and adaptation report card for Australia CSIRO publishing, Clayton, Victoria, Australia.

Ridgway, K.R. 2007, 'Long-term trend and decadal variability of the southward penetration of the East Australian Current', *Geophysical Research Letters*, vol. 34, no. 13.

Rizzo, P.J. 2003, 'Those amazing dinoflagellate chromosomes', *Cell research*, vol. 13, no. 4, pp. 215-7.

Rizzo, P.J. & Noodén, L.D. 1972, 'Chromosomal proteins in the dinoflagellate alga Gyrodinium cohnii', *Science*, vol. 176, no. 4036, pp. 796-7.

Ruvindy, R. 2019, 'The application of qPCR assays for the early detection of toxic *Alexandrium* in eastern australian waters'.

Ruvindy, R., Bolch, C.J., MacKenzie, L., Smith, K.F. & Murray, S.A. 2018, 'qPCR assays for the detection and quantification of multiple paralytic shellfish toxin-producing species of *Alexandrium*', *Frontiers in microbiology*, vol. 9, p. 3153.

Sakamoto, S., Lim, W.A., Lu, D., Dai, X., Orlova, T.Y. & Iwataki, M. 2020, 'Harmful algal blooms and associated fisheries damage in East Asia: current status and trends in China, Japan, Korea and Russia', *Harmful Algae*, p. 101787.

Sassenhagen, I., Gao, Y., Lozano-Duque, Y., Parsons, M.L., Smith, T.B. & Erdner, D.L. 2018, 'Comparison of spatial and temporal genetic differentiation in a harmful dinoflagellate species emphasizes impact of local processes', *Frontiers in Marine Science*, vol. 5, p. 393.

Schantz, E.J., Ghazarossian, V.E., Schnoes, H.K., Strong, F.M., Springer, J.P., Pezzanite, J.O. & Clardy, J. 1975, 'Structure of saxitoxin', *Journal of the American Chemical Society*, vol. 97, no. 5, pp. 1238-9.

Schilbert, H.M., Rempel, A. & Pucker, B. 2020, 'Comparison of read mapping and variant calling tools for the analysis of plant NGS data', *Plants*, vol. 9, no. 4, p. 439.

Scholin, C.A., Buck, K.R., Britschgi, T., Cangelosi, G. & Chavez, F.P. 1996, 'Identification of *Pseudo-nitzschia australis* (Bacillariophyceae) using rRNA-targeted probes in whole cell and sandwich hybridization formats', *Phycologia*, vol. 35, no. 3, pp. 190-7.

Sebastián, C.R., Etheridge, S.M., Cook, P.A., O'Ryan, C. & Pitcher, G.C.J.P. 2005, 'Phylogenetic analysis of toxic *Alexandrium* (Dinophyceae) isolates from South Africa: implications for the global phylogeography of the *Alexandrium tamarense* species complex', vol. 44, no. 1, pp. 49-60.

Selander, E., Kubanek, J., Hamberg, M., Andersson, M., Cervin, G. & Pavia, H. 2015, 'Predator lipids induce paralytic shellfish toxins in bloom-forming algae', *Proceedings of the National Academy of Sciences*, vol. 112, no. 20, pp. 6395-400.

Selander, E., Thor, P., Toth, G. & Pavia, H. 2006, 'Copepods induce paralytic shellfish toxin production in marine dinoflagellates', *Proceedings of the Royal Society B: Biological Sciences*, vol. 273, no. 1594, pp. 1673-80.

Sellner, K.G., Doucette, G.J. & Kirkpatrick, G.J. 2003, 'Harmful algal blooms: causes, impacts and detection', *Journal of Industrial Microbiology Biotechnology*, vol. 30, no. 7, pp. 383-406.

Shimizu, Y., Norte, M., Hori, A., Genenah, A. & Kobayashi, M. 1984, 'Biosynthesis of saxitoxin analogs: the unexpected pathway', *Journal of the American Chemical Society*, vol. 106, no. 21, pp. 6433-4.

Shumway, S.E. 1990, 'A review of the effects of algal blooms on shellfish and aquaculture', *Journal of the world aquaculture society*, vol. 21, no. 2, pp. 65-104.

Sjöqvist, C., Godhe, A., Jonsson, P.R., Sundqvist, L. & Kremp, A. 2015, 'Local adaptation and oceanographic connectivity patterns explain genetic differentiation of a marine diatom across the North Sea–Baltic Sea salinity gradient', *Molecular ecology*, vol. 24, no. 11, pp. 2871-85.

Smayda, T.J. 1990, *Novel and nuisance phytoplankton blooms in the sea: evidence for a global epidemic*, RWS-North Sea Directorate.

Smayda, T.J. 1997a, 'Bloom dynamics: physiology, behavior, trophic effects', *Limonology and Oceanography*, vol. 42, pp. 1132-6.

Smayda, T.J. 1997b, 'Harmful algal blooms: their ecophysiology and general relevance to phytoplankton blooms in the sea', *Limnology and Oceanography*, vol. 42, no. 5part2, pp. 1137-53.

Smayda, T.J. & Reynolds, C.S. 2003, 'Strategies of marine dinoflagellate survival and some rules of assembly', *Journal of Sea Research*, vol. 49, no. 2, pp. 95-106.

Spector, D.L. 1984, 'Dinoflagellates: an introduction', *Dinoflagellates*, pp. 1-15.

Stüken, A., Orr, R.J.S., Kellmann, R., Murray, S.A., Neilan, B.A. & Jakobsen, K.S. 2011, 'Discovery of nuclear-encoded genes for the neurotoxin saxitoxin in dinoflagellates', *PLoS One*, vol. 6, no. 5, p. e20096.

Su, Z., Sheets, M., Ishida, H., Li, F. & Barry, W.H. 2004, 'Saxitoxin blocks L-type ICa', *Journal of Pharmacology Experimental Therapeutics*, vol. 308, no. 1, pp. 324-9.

Suikkanen, S., Kremp, A., Hautala, H. & Krock, B. 2013, 'Effects of salinity on growth rate and PST/spirolide production of the dinoflagellate *Alexandrium ostenfeldii*', *Harmful Algae*, vol. 26, pp. 52-9.

Sundqvist, L., Godhe, A., Jonsson, P.R. & Sefbom, J. 2018, 'The anchoring effect—long-term dormancy and genetic population structure', *The ISME journal*, vol. 12, no. 12, pp. 2929-41.

Suthers, I.M., Young, J.W., Baird, M.E., Roughan, M., Everett, J.D., Brassington, G.B., Byrne, M., Condie, S.A., Hartog, J.R. & Hassler, C.S. 2011, 'The strengthening East Australian Current, its eddies and biological effects—an introduction and overview', *Deep-Sea Research II*.

Tang, Y.Z., Kong, L. & Holmes, M.J. 2007, 'Dinoflagellate Alexandrium leei (Dinophyceae) from Singapore coastal waters produces a water-soluble ichthyotoxin', *Marine Biology*, vol. 150, no. 4, pp. 541-9.

Taylor, F.J.R., Fukuyo, Y., Larsen, J. & Hallegraeff, G.M. 2003, 'Taxonomy of harmful dinoflagellates', in G.M. Hallegraeff, Anderson, D.M., Cembella, A.D. (ed.), *Manual on Harmful Marine Microalgae*, 2nd edn, vol. 11, IOC-UNESCO, Paris, pp. 389-432.

Tester, P.A., Litaker, R.W. & Berdalet, E. 2020, 'Climate change and harmful benthic microalgae', *Harmful algae*, vol. 91, p. 101655.

Thompson, P.A., Bonham, P., Waite, A.M., Clementson, L.A., Cherukuru, N., Hassler, C. & Doblin, M.A.D.S.R.P.I.T.S.i.O. 2011, 'Contrasting oceanographic conditions and phytoplankton communities on the east and west coasts of Australia', *Deep Sea Research Part II: Topical Studies in Oceanography*, vol. 58, no. 5, pp. 645-63.

Tillmann, U., Alpermann, T.J., John, U. & Cembella, A.D. 2008, 'Allelochemical interactions and short-term effects of the dinoflagellate Alexandrium on selected photoautotrophic and heterotrophic protists', *Harmful Algae*, vol. 7, no. 1, pp. 52-64.

Tillmann, U. & Hansen, P.J. 2009, 'Allelopathic effects of Alexandrium tamarense on other algae: evidence from mixed growth experiments', *Aquatic Microbial Ecology*, vol. 57, no. 1, pp. 101-12.

Tillmann, U. & John, U. 2002a, 'Toxic effects of *Alexandrium spp*. on heterotrophic dinoflagellates: an allelochemical defence mechanism independent of PSP-toxin content', *Marine ecology progress series*, vol. 230, pp. 47-58.

Tillmann, U. & John, U.J.M.e.p.s. 2002b, 'Toxic effects of Alexandrium spp. on heterotrophic dinoflagellates: an allelochemical defence mechanism independent of PSP-toxin content', vol. 230, pp. 47-58.

Tillmann, U., Kremp, A., Tahvanainen, P. & Krock, B. 2014, 'Characterization of spirolide producing Alexandrium ostenfeldii (Dinophyceae) from the western Arctic', *Harmful Algae*, vol. 39, pp. 259-70.

Tomas, C.R., van Wagoner, R., Tatters, A.O., White, K.D., Hall, S. & Wright, J. 2012, '*Alexandrium peruvianum* (Balech and Mendiola) Balech and Tangen a new toxic species for coastal North Carolina', *Harmful Algae*, vol. 17, pp. 54-63.

Touzet, N., Franco, J.M. & Raine, R. 2007, 'Characterization of nontoxic and toxin-producing strains of *Alexandrium minutum* (Dinophyceae) in Irish coastal waters', *Applied Environmental Microbiology*, vol. 73, no. 10, pp. 3333-42.

Townsend, D.W., Pettigrew, N.R. & Thomas, A.C. 2001, 'Offshore blooms of the red tide dinoflagellate, *Alexandrium* sp., in the Gulf of Maine', *Continental Shelf Research*, vol. 21, no. 4, pp. 347-69.

Trainer, V.L., Moore, S.K., Hallegraeff, G., Kudela, R.M., Clement, A., Mardones, J.I. & Cochlan, W.P. 2020, 'Pelagic harmful algal blooms and climate change: lessons from nature's experiments with extremes', *Harmful algae*, vol. 91, p. 101591.

Tsuchiya, S., Cho, Y., Yoshioka, R., Konoki, K., Nagasawa, K., Oshima, Y. & Yotsu-Yamashita, M. 2017, 'Synthesis and identification of key biosynthetic intermediates for the formation of the tricyclic skeleton of saxitoxin', *Angewandte Chemie*, vol. 129, no. 19, pp. 5411-5.

Turnbull, A., Malhi, N., Seger, A., Harwood, T., Jolley, J., Fitzgibbon, Q. & Hallegraeff, G. 2020, 'Paralytic shellfish toxin uptake, tissue distribution, and depuration in the Southern Rock Lobster Jasus edwardsii Hutton', *Harmful Algae*, vol. 95, p. 101818.

Usup, G., Ahmad, A., Matsuoka, K., Lim, P.T. & Leaw, C.P. 2012, 'Biology, ecology and bloom dynamics of the toxic marine dinoflagellate *Pyrodinium bahamense*', *Harmful Algae*, vol. 14, pp. 301-12.

Usup, G., Kulis, D.M. & Anderson, D.M. 1994, 'Growth and toxin production of the toxic dinoflagellate *Pyrodinium bahamense* var. *compressum* in laboratory cultures', *Natural toxins*, vol. 2, no. 5, pp. 254-62.

Usup, G., Pin, L.C., Ahmad, A. & Teen, L.P. 2002, '*Alexandrium* (Dinophyceae) species in Malaysian waters', *Harmful Algae*, vol. 1, no. 3, pp. 265-75.

Valério, E., Chaves, S. & Tenreiro, R. 2010, 'Diversity and impact of prokaryotic toxins on aquatic environments: a review', *Toxins*, vol. 2, no. 10, pp. 2359-410.

Van Dolah, F.M., Lidie, K.B., Morey, J.S., Brunelle, S.A., Ryan, J.C., Monroe, E.A. & Haynes, B.L. 2007, 'Microarray analysis of diurnal-and circadian-regulated genes in the florida red-tide dinoflagellate *Karenia brevis* (Dinophyceae) 1', *Journal of Phycology*, vol. 43, no. 4, pp. 741-52.

Verma, A., Barua, A., Ruvindy, R., Savela, H., Ajani, P.A. & Murray, S.A. 2019, 'The genetic basis of toxin biosynthesis in dinoflagellates', *Microorganisms*, vol. 7, no. 8, p. 222.

Verma, A., Hughes, D.J., Harwood, D.T., Suggett, D.J., Ralph, P.J. & Murray, S.A. 2020, 'Functional significance of phylogeographic structure in a toxic benthic marine microbial eukaryote over a latitudinal gradient along the East Australian Current', *Ecology and Evolution*.

Visciano, P., Schirone, M., Berti, M., Milandri, A., Tofalo, R. & Suzzi, G. 2016, 'Marine biotoxins: occurrence, toxicity, regulatory limits and reference methods', *Frontiers in microbiology*, vol. 7, p. 1051.

Waggett, R.J., Tester, P.A. & Place, A.R. 2008, 'Anti-grazing properties of the toxic dinoflagellate *Karlodinium veneficum* during predator–prey interactions with the copepod Acartia tonsa', *Marine Ecology Progress Series*, vol. 366, pp. 31-42.

Wang, D. 2008, 'Neurotoxins from marine dinoflagellates: a brief review', *Marine Drugs*, vol. 6, no. 2, pp. 349-71.

Wang, D. & Hsieh, D.P.H. 2002, 'Effects of nitrate and phosphate on growth and C2 toxin productivity of *Alexandrium tamarense* CI01 in culture', *Marine pollution bulletin*, vol. 45, no. 1-12, pp. 286-9.

Wang, H., Tomasch, J., Jarek, M. & Wagner-Döbler, I. 2014, 'A dual-species co-cultivation system to study the interactions between Roseobacters and dinoflagellates', *Frontiers in microbiology*, vol. 5, p. 311.

Wang, J., Salata, J.J. & Bennett, P.B. 2003, 'Saxitoxin is a gating modifier of HERG K+ channels', *The Journal of General Physiology*, vol. 121, no. 6, pp. 583-98.

Wells, M.L., Trainer, V.L., Smayda, T.J., Karlson, B.S., Trick, C.G., Kudela, R.M., Ishikawa, A., Bernard, S., Wulff, A. & Anderson, D.M. 2015, 'Harmful algal blooms and climate change: Learning from the past and present to forecast the future', *Harmful algae*, vol. 49, pp. 68-93.

Whittaker, K.A. & Rynearson, T.A. 2017, 'Evidence for environmental and ecological selection in a microbe with no geographic limits to gene flow', *Proceedings of the National Academy of Sciences*, vol. 114, no. 10, pp. 2651-6.

Wickham, H. 2016a, *ggplot2: Elegant Graphics for Data Analysis*, Springer-Verlag New York.

Wickham, H. 2016b, 'tidyverse:Easily Install and Load "Tidyverse" Packages.', *R package version 1.0.0.*

Wiese, M., D'agostino, P.M., Mihali, T.K., Moffitt, M.C. & Neilan, B.A. 2010, 'Neurotoxic alkaloids: saxitoxin and its analogs', *Marine drugs*, vol. 8, no. 7, pp. 2185-211.

Wiese, M., Murray, S.A., Alvin, A. & Neilan, B.A. 2014, 'Gene expression and molecular evolution of sxtA4 in a saxitoxin producing dinoflagellate Alexandrium catenella', *Toxicon*, vol. 92, pp. 102-12.

Wohlrab, S., Iversen, M.H. & John, U. 2010, 'A molecular and co-evolutionary context for grazer induced toxin production in Alexandrium tamarense', *PLoS One*, vol. 5, no. 11, p. e15039.

Wohlrab, S., Selander, E. & John, U. 2017, 'Predator cues reduce intraspecific trait variability in a marine dinoflagellate', *BMC ecology*, vol. 17, no. 1, p. 8.

Wolfe, G.V., Levasseur, M., Cantin, G. & Michaud, S. 2000, 'DMSP and DMS dynamics and microzooplankton grazing in the Labrador Sea: application of the dilution technique', *Deep Sea Research Part I: Oceanographic Research Papers*, vol. 47, no. 12, pp. 2243-64.

Wong, J.T.Y., New, D.C., Wong, J.C.W. & Hung, V.K.L. 2003, 'Histone-like proteins of the dinoflagellate *Crypthecodinium cohnii* have homologies to bacterial DNA-binding proteins', *Eukaryotic Cell*, vol. 2, no. 3, pp. 646-50.

Yang, I., John, U., Beszteri, S., Glöckner, G., Krock, B., Goesmann, A. & Cembella, A.D. 2010, 'Comparative gene expression in toxic versus non-toxic strains of the marine dinoflagellate *Alexandrium minutum*', *BMC genomics*, vol. 11, no. 1, p. 248.

Yang, I., Selander, E., Pavia, H. & John, U. 2011, 'Grazer-induced toxin formation in dinoflagellates: a transcriptomic model study', *European Journal of Phycology*, vol. 46, no. 1, pp. 66-73.

Yasumoto, T. & Murata, M. 1993, 'Marine toxins', *Chemical reviews*, vol. 93, no. 5, pp. 1897-909.

Yñiguez, A.T. & Ottong, Z.J. 2020, 'Predicting fish kills and toxic blooms in an intensive mariculture site in the Philippines using a machine learning model', *Science of The Total Environment*, vol. 707, p. 136173.

Zhang, H., Bhattacharya, D. & Lin, S. 2007, 'A three-gene dinoflagellate phylogeny suggests monophyly of prorocentrales and a basal position for *Amphidinium* and *Heterocapsa*', *Journal of Molecular Evolution*, vol. 65, no. 4, pp. 463-74.

Zhang, S.F., Zhang, Y., Lin, L. & Wang, D.Z. 2018, 'iTRAQ-based quantitative proteomic analysis of a toxigenic dinoflagellate *Alexandrium catenella* and its non-toxigenic mutant exposed to a cell cycle inhibitor colchicine', *Frontiers in microbiology*, vol. 9, p. 650.

Zhang, Y., Zhang, S.F., Lin, L. & Wang, D.Z. 2014, 'Comparative transcriptome analysis of a toxin-producing dinoflagellate *Alexandrium catenella* and its non-toxic mutant', *Marine drugs*, vol. 12, no. 11, pp. 5698-718.

Zhang, Z., Green, B.R. & Cavalier-Smith, T. 1999, 'Single gene circles in dinoflagellate chloroplast genomes', *Nature* vol. 400, no. 6740, pp. 155-9.

Zhao, Y., Wang, K., Wang, W.l., Yin, T.t., Dong, W. & Xu, C. 2019, 'A high-throughput SNP discovery strategy for RNA-seq data', *BMC genomics*, vol. 20, no. 1, p. 160.

Zhou, J., Richlen, M.L., Sehein, T.R., Kulis, D.M., Anderson, D.M. & Cai, Z. 2018, 'Microbial community structure and associations during a marine dinoflagellate bloom', *Frontiers in microbiology*, vol. 9, p. 1201.

Supplementary Document

Fig. S1A. Standard curve developed from *A. australiense* strain AT-YC-H

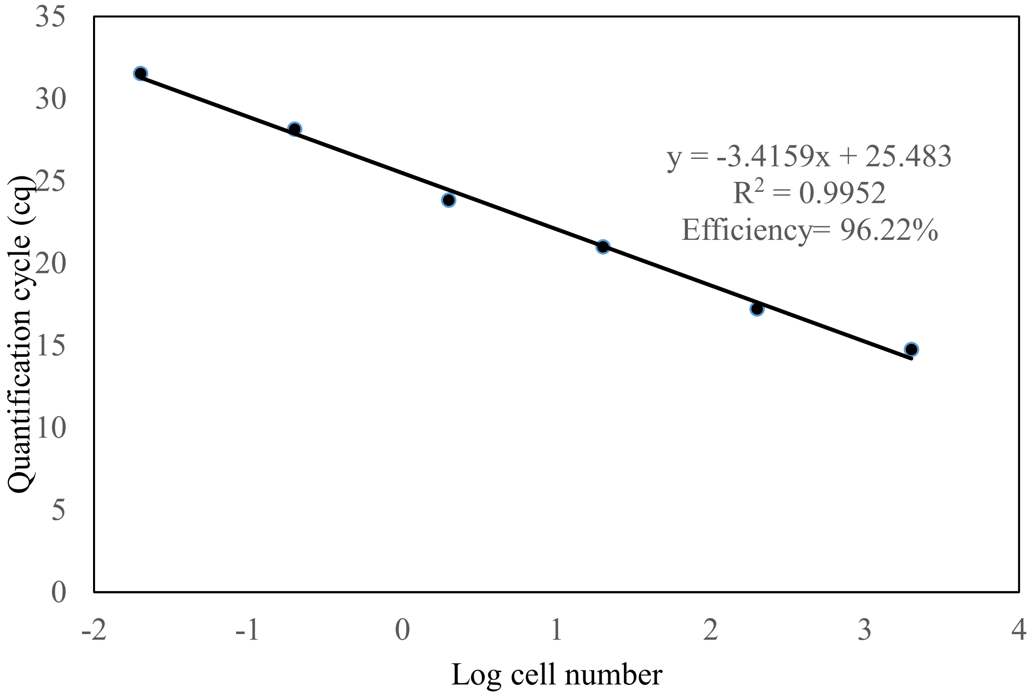


Fig. S1 B. Standard curve developed from *A. pacificum* strain CS300

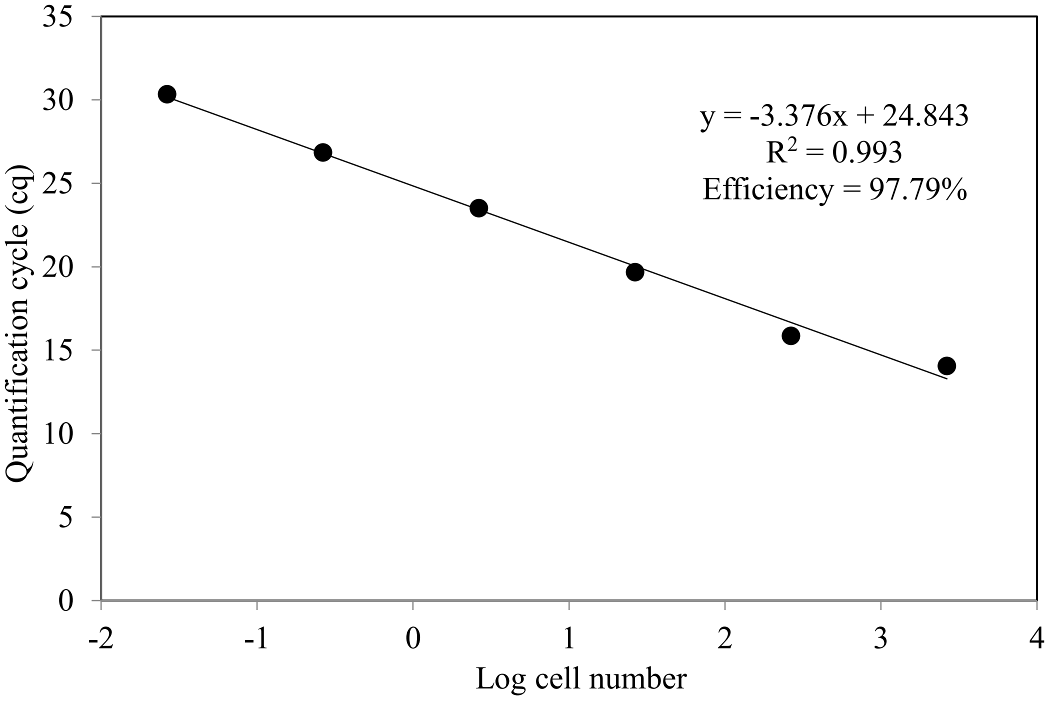


Table S1: Phytoplankton action limits (PALs) for potential PST-producing species in NSW shellfish aquaculture areas. (NSW MBMP, 2016)

Phytoplankton species	**Trigger flesh sampling (cells/L)**	**Alert level – close harvest area pending flesh testing results**	**Issue public health warning (cells/L)**
*Alexandrium minutum**	200	500	5,000
*Alexandrium ostenfeldii**	200	500	5,000
*Alexandrium catenella**	200	500	5,000
*Alexandrium tamarense**	200	500	5,000
Alexandrium spp.*			
Gymnodinium catenatum	1000 mussels 2000 other shellfish	5,000	5,000

**Alexandrium* species may be difficult to identify when numbers are low. If any doubt exists, they should be treated as potentially toxic.

Table S2: Procedures for changes to harvest status in classified NSW shellfish harvest areas when potentially harmful phytoplankton species above phytoplankton action limits (PAL) in seawater and algal biotoxins in shellfish flesh are detected. (NSW MBMP, 2016)

Harvest area status	**Phytoplankton (cells/L)**	**Biotoxin (mg/kg)**
Open (routine monitoring)	Cells/L < PAL (fortnightly)	Negative result (monthly)
Open (increased monitoring frequency)	Cells/L > sampling PAL (weekly)	Positive result < regulatory limit (weekly)
Closed*	Cells/L > closure PAL or Sample not reported to schedule	Positive result > regulatory limit or Sample not reported to schedule

Table S3 Amplicon sequencing abundance data from samples collected on 24 November 2016

id	Phylum	S1	S2	S3	S4	S5	S6
09dcd416a785aed8f55273922a157cde	Dinoflagellata	7052	11872	199654	154796	15463	371
3c0426ff00a13898dcb5a4dd538345f8	Dinoflagellata	5167	7454	153147	131094	12534	272
74d66cdac8ad8046675d93cc81f90d03	Dinoflagellata	6308	7754	129951	123423	19003	317
a04b87608e2861e56068b2ec01bf7050	Dinoflagellata	1371	1920	120483	153868	12781	270
902cfc0375eb227155a6d1d5b59f92ae	Dinoflagellata	5170	6803	156936	106578	9339	247
bf8bb165e5e3f76cfbc0a90fc35439dd	Dinoflagellata	6107	9653	110473	70441	13946	361
1c22f8f84b75a9dc09e2baf2749139e6	Dinoflagellata	4914	6067	71284	74693	23974	375
2759e0ba8b73e16ffb54356571ae269d	Dinoflagellata	952	1156	43977	76320	18591	262
1d163eef9e96e3522fcc02a4f8b7a158	Dinoflagellata	4265	5174	63124	52262	13954	254
3354cb01df148dbe6972028c4050da44	Ciliophora	12399	861	97	81	0	
b7f2e1f0f14a023912d3e995168a2a19	Metazoa	8969	950	1183	1656	111	
3565b6fe34510699c287a3f457a3f334	Ciliophora	11502	776	130	149	0	
7333fdce061aab428ca5017793cb7088	Metazoa	7812	802	1235	2383	116	
05b4d943f698c1bb68edada2abead4f0	Ciliophora	9159	565	71	72	19	
f45336eba9f271d8bb615846667e4c19	Metazoa	0	0	2974	0	16	15
dd5f3751dd9f1c9c8ba2fdf445b7a650	Dinoflagellata	73	103	2342	1465	144	3
302af4f91082fb3f8ac6bc0397515bea	Metazoa	0	0	3306	0	16	11
c727d6e724fd1d1e8e59db2e65418ef0	Dinoflagellata	71	99	1963	1522	154	3
4d443b2765bc6574566c552b8db1466a	NA	1274	1529	0	0	0	
1d36229189022f562a1cbb35926cac3f	Dinoflagellata	31	66	1266	879	95	2
6d8297d44aefab37719d5a54dcd73dc7	Dinoflagellata	41	70	1210	907	73	2
9d75c182429d8a1f64c5e68e15e3e19a	NA	1554	258	233	286	31	1
99d9d4958279b9f1a31d91ed652b6037	Dinoflagellata	55	90	988	756	216	4
4b1373f4bf4d4938323e8d40d5461729	NA	1494	275	227	326	8	1
76f2c132f2a4b1535878a8197ce81aa6	Ochrophyta	70	1653	55	271	76	2
1736f8d4eb7fb96994a0b2339eb78184	Ochrophyta	54	1372	55	368	45	1
49e2adb57b4c6e112c3366191982ebd9	Dinoflagellata	0	52	1026	744	50	1
dd6b416321b2202eabe1dc5e6687a9ec	Dinoflagellata	64	138	910	405	60	2
051abe3c76e3e4ca01c2e2b2a6f965d1	Metazoa	0	1399	0	0	0	
2a3939eeaaa7dd5409c6233534d9a4a6	Cercozoa	0	1008	0	123	0	1
99a14f4a8a1650ac5fa3abb6653772b1	Dinoflagellata	37	35	375	446	134	2
7558578df2d766070859bde200887bda	Metazoa	0	0	1120	0	0	
86c7c1c80ebf8f8add0c2a0840d73a76	Dinoflagellata	1046	0	0	40	0	
2b20600c419d2aba8eeba6918904fdb1	Dinoflagellata	0	25	440	282	39	3
a459f13d10be82a89ae2e5e925c354cf	Dinoflagellata	14	12	414	386	36	
1aa91d083f50b7e8fd6ae5d033cd3810	Cryptophyta	34	284	0	416	0	1
f6c156dfb4b75e319e1a845271b02370	Cercozoa	160	0	0	0	630	
82b65b0135a7f347b39e4732fe5500c0	NA	759	0	0	0	0	
43ae3d16f457a0a6f53a66a8876b8e18	Haptophyta	34	361	71	115	0	1
e8a9fb3ad43d6a99af2c03dbad835c9b	Dinoflagellata	0	0	393	297	0	
483329c5b39abb436a71fc5bcdb66efc	Stramenopiles_X	34	377	0	90	0	1
520db09e704b31405a3eb5fc8d4c3b9c	NA	570	0	0	0	0	
5541a34bc769d51f613577b295df885f	Ochrophyta	128	65	42	22	245	
63c23ca11920630adcbdf658e547f363	Dinoflagellata	63	42	77	49	276	

5e2cdb8666f1059b5c6ac620f46c2c8e	Cercozoa	92	203	0	94	0
f6f54b349532784c2dd7965f4e961bbd	Dinoflagellata	13	12	174	163	49
d2db4299fae956b6f79f511e0d1f4b76	NA	0	71	102	125	98
aca6ce7542f05ab846ed28ef61d2e0cd	Dinoflagellata	56	20	141	53	187
41066bbfd9d4526369e91fb00b2d6dcd	Metazoa	0	0	0	0	476
09201978b733bf7a7034cdc80c9f32bd	Stramenopiles_X	38	172	0	150	0
ab761f11facfb9efc2e1197fb5cba259	Metazoa	0	91	381	0	0
7f33173d234b9b22c6b8ce95a80601b5	Metazoa	0	0	0	455	0
26b21b9b8513db0b447d80d3db8478dc	NA	0	103	101	157	0
18932291751d0f21de12b4609e7e53b8	Dinoflagellata	334	28	0	67	0
4ed4825990216b3b103d52820f22e636	Dinoflagellata	0	0	0	412	0
91c988af78fded2210c437066cb53342	NA	253	32	36	65	0
0f55c33dafe5083bd5a289fcb2489dab	Dinoflagellata	202	0	84	64	0
bfe919ed2a31bce1fb8a154bc48d4f64	NA	255	37	55	47	0
6f163c3579474b3e1e3d4ce20fec7798	Ciliophora	218	94	0	51	0
336f0cea960385745a14a1b254ab8480	NA	393	0	0	0	0
5ce64f2716e17b92aeb2480fb91e418b	Ochrophyta	76	97	52	40	76
c1aeff410783063b2b14f2eedb2a5b78	Dinoflagellata	38	0	171	121	9
68a063e1d32a56b2450d8c9045279a6d	Dinoflagellata	0	0	0	361	0
cd2355d45d0cb9ca4c9211c3368b0f00	NA	157	198	0	0	0
33f454109a6d3ccd6b825c6657370fe2	Ochrophyta	64	57	62	38	78
cd4ecfd64edcb7083ae93c546d4a6013	Ciliophora	0	134	0	44	0
044ac041f2d229fc563122b418f0f4e5	Metazoa	0	0	0	0	319
7743b96eecf7b68f7206160f6f38027e	Ochrophyta	0	0	0	0	314
4c1585305979510e7591b2520bfe5b6e	Dinoflagellata	0	49	0	148	0
eb37c50aa93c60a2fa71009a9089f2e6	NA	204	26	16	27	0
8d6ceee9b4570f10369bfa9643f6cb53	Chlorophyta	28	192	0	60	0
ba3c6229316bb8ec4268ac9dc3f52206	Dinoflagellata	0	121	0	125	0
e8baf45b587e26b0dd29c36e4c573b00	Ochrophyta	110	45	25	17	97
8898b4791c21fd557239b9f14044fce6	Dinoflagellata	88	14	86	12	48
f0db2e3df79407f1e075d853b9ab9e79	Mesomycetozoa	0	0	13	0	255
5d8e079e33742968f49db9c5b43fd577	NA	0	0	75	114	41
27a987bdd34f5909c9966220f57500fe	Ochrophyta	94	0	36	49	60
1185befb485175c398e1f0e5aafb3a50	Metazoa	266	0	0	0	0
c7bff6f8fa76e988102aaa190eb82e16	Haptophyta	0	149	38	79	0
64573a6c2e1c9c5e8cc23cec4e291593	Haptophyta	0	74	0	96	0
c9ee5b4f6118b1ab16a35ef3e92c0d3b	Cercozoa	0	111	0	69	0
7f1b72d686374cb6761d2ad2f1667701	Dinoflagellata	102	10	18	0	109
6867555a92a54afc9c9f6cf1b486400f	Katablepharidophyta	3	159	0	35	0
78a9757aff0af66534f4482c36e5ce2f	NA	0	0	43	83	64
db59cd17e857ef0b99f910ddffbea67b	Metazoa	0	0	0	0	242
f27e83a1dde937c857d3e5580b0a53ad	Dinoflagellata	116	12	25	0	84
7ae47f04907386b0b017e347573680e6	Dinoflagellata	0	0	240	0	0
541d95309feb8846a7aa3bcba508bc76	Dinoflagellata	0	0	237	0	0
95fd9cbbc09a723e89c327fab28b7cf1	Haptophyta	0	113	0	58	0
13e6c53549a3199da9a3d74184ad5885	Dinoflagellata	0	0	117	45	40

7524b12023d75031f08f812f60d96d75	Cryptophyta	0	86	0	91	0
d10e591a655f505bde6153de85e4dd19	Stramenopiles_X	15	87	0	70	0
7c9fb430e7d9569c4fd0e6fe8960f35d	Stramenopiles_X	29	72	0	0	45
8f2fd08621ac51b7f9416f0c86aae073	Dinoflagellata	0	77	0	61	0
6f41b29d85471549fd8ba425d24aaff8	Fungi	96	71	0	32	0
a8eccfc81f74d7bc059d078bdbdbb8eb	Haptophyta	0	112	0	30	0
461dece6e81cb6c254b934937b5957f1	Dinoflagellata	32	0	78	36	0
4436ae975509e9ea7ef286b96a855de1	Katablepharidophyta	0	66	0	85	0
5f28514ced1b02cadb0c5f6b85a010c0	Dinoflagellata	0	0	211	0	0
30fbf14dd02e855d980f3377fd242693	Stramenopiles_X	0	108	0	36	0
69af9f48e60fece7b59d4423a9ab0227	Dinoflagellata	0	0	139	0	0
40c0baa05e8714c6ecae70053dddf208	NA	0	0	0	85	78
b5b82bcc2e6d4def1d93f671cf9e8943	Radiolaria	174	33	0	0	0
a3ec69e455e77147f1f361b837ba48e0	NA	27	51	37	51	39
418de0667f91ad67909e9e74296a05c2	Chlorophyta	37	89	0	36	0
3c550f5b78b041271325e0ce6e3fb912	Dinoflagellata	0	0	76	48	0
36331cf584f9310b5e6598d2db9bd702	Ochrophyta	47	73	0	28	28
9c4df517ff3e7e23080ca8395cb8a055	Haptophyta	0	84	0	59	0
1127ae90588df95925aed1acbfae04d8	Haptophyta	0	94	0	50	0
83961e7b6e519feff5f09464aba6a81f	Dinoflagellata	0	0	48	103	0
ad96aeccac995f789327924c6b8d580e	Dinoflagellata	43	0	81	65	0
c23f7dd5b0f6004202ce425436f33b8a	Cryptophyta	0	0	0	189	0
12922a1d2fbe8cf7587b610ccad1f321	Dinoflagellata	0	0	88	44	31
b0c9ee7b7741b2a439db7fc05130aa6c	Dinoflagellata	0	68	39	39	0
16a1e7184ca921ff7d9314f9be317e7a	Metazoa	179	0	0	0	0
98bbf3ee4ccdde641261e50d19b6cf3a	Cercozoa	0	120	0	37	0
3f5b44446ca62562d9986076f9862ddb	Chlorophyta	47	69	0	19	0
c30e44d4a2f4f92deefa492567abf17e	Stramenopiles_X	47	64	0	0	63
9216adacbd97deed5bd4e7b538021b7c	Cryptophyta	0	17	0	105	0
2182fec9e376049c037384e8bce4b5ef	Chlorophyta	8	102	0	21	0
5b1de6497d1cdad688674756d1302c7f	Dinoflagellata	0	0	72	44	0
2fddf5b020db7cc46450dbd5562ecac1	Cercozoa	0	111	0	34	0
2ae55ad075e0982b0c3a450435e5ab35	Dinoflagellata	0	0	53	0	50
eba87926c7b0baa32cd1d00034c42078	Dinoflagellata	0	144	0	0	0
0511a05cebf70653a89d95344554a905	Haptophyta	0	71	0	36	0
1991c98d3ce663733f138a10f26ad1ab	Dinoflagellata	0	46	0	58	0
077de4eb0d58481a17aa1551d1bab314	Dinoflagellata	0	67	0	47	0
28ba387bbaadfc60a50230544ab5db91	Picozoa	0	40	0	62	0
b58ca4fb781101409055d60f9a2577e8	Eukaryota_XX	0	109	0	41	0
526913963a89c9731ccded87321c10af	Dinoflagellata	0	0	66	49	0
3b5f426eaa18de6fcaadc8269d81797e	Dinoflagellata	0	25	0	73	0
83387b829c9d698037e4f114ba1127de	Dinoflagellata	0	54	0	40	0
d89bae10fb370513855d010513aff50d	Dinoflagellata	0	37	0	64	0
cd1dac3015606d92a62e2f3f697e8763	Dinoflagellata	0	0	41	69	0
e9f48b61a955fa83abafd35117508c10	Dinoflagellata	0	33	0	78	0
74cf32c39361b33815bafd638ec7f845	Metazoa	0	0	143	0	0

652cd51b4a7a156fca517f673d41bbfa	Haptophyta	0	71	0	0	0
e99fc9e97a9d0585b98f5268ad24017a	NA	0	0	0	92	0
fae944e96a5608388bf3f9b7f7966caa	Dinoflagellata	0	59	0	60	0
87e555bb8f86cf35e4945c56c67eeded	Dinoflagellata	0	47	0	56	0
21b116cfb59ae86cd0cd314c0ade6dff	Dinoflagellata	65	12	0	0	58
9a6abdb38a537dfbdb88092d3cc6a098	Ciliophora	0	0	0	0	133
0f8dc3bb852cf229a47c17a88785c327	Ochrophyta	0	51	0	8	0
18f54f6219a44e123f115e8e8f9ad0d0	Ochrophyta	65	62	0	0	0
a84d9be6ca817265dbd01c2829eef6cd	Dinoflagellata	71	0	0	55	0
430e08ca7e155980eb3b9ff7b8a7dd9c	Dinoflagellata	0	0	36	46	0
87c94422e4179eb475a8bce66788d0ec5	Dinoflagellata	0	0	35	65	0
b63050e28efe81c45f83b75ded7ed01b	Ciliophora	0	49	0	0	0
09e1d3a1e481d5d1e78aef9f1e80a012	Metazoa	0	0	0	0	121
72d37cedfc3016805fabbbbe746d41cf	Dinoflagellata	0	31	0	64	0
f918bf78780f962a98524d99690c7f5c	Dinoflagellata	0	0	0	89	0
66762c49b0c2a5ecf1be934fb0437f91	Cercozoa	0	61	0	0	0
b42c074ec0af6fec3112872e5e78dc85	Dinoflagellata	0	70	0	45	0
c3cbc9176d1aee3657c0de39cb55f925	Stramenopiles_X	0	56	0	0	0
00602f544f0049a23a263349d1508ba2	Dinoflagellata	113	0	0	0	0
c8e788bfa0155ee4ef1e14bd494d02c4	Picozoa	0	58	0	55	0
8901a3472c6b7cabb3afa7f403e6e8b7	Chlorophyta	27	50	0	7	0
9edc33bbd9edba794df54a276432d783	Dinoflagellata	0	73	0	38	0
be29615a88d14a8307f4e9a6765f905d	Apicomplexa	0	48	0	36	0
419a7a325d071ec7286b0530c3f1ffce	Dinoflagellata	0	27	20	43	0
090527ea82660e0cd7d42864238ac341	Cercozoa	38	71	0	0	0
4b5892bd537bfa6f6ba27577b335b6f2	Metazoa	0	0	0	0	109
499cc167fd5088f467969a6b5b110303	Dinoflagellata	0	46	0	61	0
26913128e3dd0d63ecb0d161e703fc23	Chlorophyta	22	45	0	20	0
0f42e2862ad1a181fb2d4b3ce8af8aba	Dinoflagellata	0	0	0	61	0
95291a8aca34cd2a31ca685c2b7c3889	Cryptophyta	0	0	0	103	0
e478af5b0d4bcef9006573723609b25c	Dinoflagellata	102	0	0	0	0
eecbccc2ded64c69f03d1c077398ce71	Dinoflagellata	0	58	0	44	0
205d76fcaf75006f4662063543702bf5	NA	101	0	0	0	0
04c1068f425d1a77068bf93e78d27fa2	NA	68	32	0	0	0
c284a6dbaf6c0f575e63b1aa16f29eb7	Dinoflagellata	0	23	32	36	0
43eea815a3c5f405f968bf6c29332ce3	Haptophyta	20	0	21	23	0
4790850852b54d850a8e9945ff0abe69	Ochrophyta	55	18	0	25	0
9058625ad82dd3a41e9a4405df485e2c	Ciliophora	0	45	0	0	0
e48118a6d86813b905594464356d03df	Mesomycetozoa	0	0	0	0	98
0e90356848ac788807721e76cc2ad2e5	Dinoflagellata	0	0	0	58	0
56c4436e10c96e40c4fd652a9e60414d	NA	95	0	0	0	0
86436f9b9fb8689f3d6793f11a1fa53c	Dinoflagellata	0	40	0	20	0
da7f2cb1e14e12fb31d7f0e3114cd987	Chlorophyta	12	18	0	48	16
4b801f2bd3ef8ed4a3553c76c80f7655	Dinoflagellata	0	32	0	0	0
fa8824eb1e13289f63d4a34860e8602a	Dinoflagellata	0	0	68	26	0
318494e71fdeab5dde0a63fc029841a6	NA	0	0	0	37	0

567f9ee9cff10b5e0b7fd1d9cbd7f0b7	Chlorophyta	0	0	0	23	0
53c94e3c66fe8ef610ea10487f8d6858	Dinoflagellata	38	0	0	0	53
ca1ff67c51ea175d47d631c0daf4ba44	NA	37	0	35	0	19
9a278208807fd07730f4ad0033c8eae7	NA	28	34	0	0	0
75b02db051af1b3ed28365eb7feeebbe	Dinoflagellata	90	0	0	0	0
b8ffe4b72ba2f84c26974fb25acb7a78	Chlorophyta	12	44	0	20	0
808dd12c3a01cc9a3bcee9013a710f8c	Dinoflagellata	0	18	0	27	0
68eced5424a79f45301ec797806a239f	NA	0	0	0	45	44
d7ce1edbbd6f98d7e02b1b6f50c48cf4	Dinoflagellata	0	86	0	0	0
b160ba25db42e3f8a7004987fce6d0a6	Dinoflagellata	0	49	0	37	0
b1431fa514119449285f4c262f4e2c3e	Stramenopiles_X	0	21	0	37	0
58cf622cc43f600192a426876a9aedfd	Dinoflagellata	0	0	0	41	45
5c454b12715ce30645268bc493218a75	Dinoflagellata	0	85	0	0	0
a2406f0594f741d395b6b7c6b64f8223	NA	0	85	0	0	0
8cfeca599e4e98e251405d66933d46d6	Dinoflagellata	0	55	0	0	0
b35ea192edb1b4aa688d42dd5e9af322	Cryptophyta	0	0	0	84	0
453c98698db542cb761d508ccc3f077c	NA	0	0	0	84	0
3bb9091fffd4c5e3da0e1c64630c2fd6	Ciliophora	0	0	0	35	0
4a40f4af30ebea6dc4a6b1e24c222e4c	Dinoflagellata	0	0	0	0	19
e9703f2301399b5de0b5228d65e9b116	Haptophyta	0	49	0	0	0
2c2c4472b2eb1bae0193ca415e620847	Dinoflagellata	0	37	0	46	0
2efbe9f618b87c807b12a2c472d32727	Metazoa	0	0	0	0	83
453c25dcb981dcd4007a220b3c704600	Haptophyta	0	33	0	0	0
28404d744d5d4aac08375e56b0121618	Dinoflagellata	0	0	0	81	0
4e87dd958742e1420026bc4230963748	Ochrophyta	17	0	23	8	0
10407dc8bebf26f311f58f61099c21e6	Ciliophora	0	0	0	0	80
87b7c33be0341c1dc2e1ea1a16b052a6	Ciliophora	0	29	0	31	0
ecb52d8a47ceb90e75209e907ebd321d	NA	0	0	79	0	0
85b59d6f7030d7f1233579d53973c4e5	Dinoflagellata	0	0	0	0	0
a96abddb0c029f62811cd42026e24622	Dinoflagellata	0	0	0	0	0
4362c572cb027752f2f1ef8db6dc5290	Dinoflagellata	78	0	0	0	0
66d4994f7b46249f6df4b894ee723b7b	Metazoa	0	0	0	0	78
2d5f8a4ed5cbc7009655d18bb95f81d1	Chlorophyta	0	77	0	0	0
a221e81318b1e2a8c8892c8460d83c09	Ciliophora	0	0	35	10	0
5ced098ea87153da543494865b53f687	Dinoflagellata	0	0	76	0	0
0c228fd0f8600b078a93ad555c6c2c3a	NA	0	0	39	37	0
4cb98777aba76375859a6959ace2f955	Eukaryota_XX	75	0	0	0	0
ec922a41fa4c70f8173cbd6fa5f8cde1	Dinoflagellata	75	0	0	0	0
76176607e13fafb02f2bf59fd89beb03	Dinoflagellata	75	0	0	0	0
2027c0c47fa8f1f8f76f8bd89bc86442	Dinoflagellata	0	0	31	37	0
bfe7a1c8cfea5ba17a1290adea5b1dda	Dinoflagellata	0	0	0	75	0
3bbc9fc8628cc9d764a092c4912f9522	Dinoflagellata	73	0	0	0	0
ff4d39bf6a40b44cd465a5e75786c1dd	Dinoflagellata	0	0	0	73	0
166b7c3cd136e5129d541eb8494ea134	Dinoflagellata	0	0	0	73	0
d964f4f0afc21254dea9b71a3a021c96	Dinoflagellata	0	0	0	34	0
50ac9077e6b6a6854372501e7ba0e92a	Metazoa	0	0	0	0	73

e8dd44510d7b6d9a3d07cfb9c2013135	Dinoflagellata	0	0	0	0	0
a589476bb4d5a92f36d7ad53cb3b23e6	Dinoflagellata	71	0	0	0	0
0798410435c7f67a2f01bdab2ad121c2	NA	0	0	0	0	71
64c00f063fa292d24a7c601089e07ab1	Chlorophyta	0	0	0	0	40
73d39c54f60af1c7084394f178e5ee2d	NA	36	34	0	0	0
9c71cf23cbbdb932f766acdb6215c800	Chlorophyta	0	32	0	20	0
021cc73631026e62b24d321993eb29d8	Dinoflagellata	0	0	70	0	0
c6d9203aa980fb451d3b47cf34cf629d	Dinoflagellata	0	0	0	34	0
5aef862832604e64e269747adc3050e1	Dinoflagellata	0	0	69	0	0
a949e19bf74d06075bfdf517dc138c6f	NA	68	0	0	0	0
b29e4a81cac70df4b25e1d63d95edd08	Dinoflagellata	59	0	0	0	0
60766402c909c4b927ab8c80223beb86	Radiolaria	50	18	0	0	0
b5abe0a7cb18a0b20590f77f1ec41a45	Fungi	0	24	0	0	0
58c45d3019e87cf0f2aeb3fb12189d7a	Dinoflagellata	0	23	0	45	0
0f3394da727f3908dd3cf3a46cc86479	Dinoflagellata	0	0	0	0	68
419b3569831a49bdbf9410f39357e8a3	Dinoflagellata	67	0	0	0	0
55ee555690791a36be6de8f5da1db939	Dinoflagellata	0	67	0	0	0
4a5da0c47eca688c4820508917e6cb83	Ciliophora	0	24	0	43	0
0dbf4274a9b56068ff58eaa8fdaab176	Telonemia	0	0	0	67	0
38259cb04e51f0cd6d1e40de6f8e34ce	Katablepharidophyta	0	0	0	67	0
67fbd52b1bf58f0b1a30f38a0e099e70	Dinoflagellata	66	0	0	0	0
827b3930fa0fc9f19262c67f9fb3ca93	Ochrophyta	11	0	20	12	23
d99bed4a29de8ab92700337d72f6d9e1	Dinoflagellata	0	47	4	0	0
63119ab7101c0cddf4fb762dcbaf87c0	Ciliophora	0	65	0	0	0
c6b0b3b5b01bbc2bf4436245d2c62385	Ochrophyta	0	9	37	19	0
63b5eff98537490757bbf89daba82653	Dinoflagellata	0	0	22	25	0
b911533094dd9d826d53a31df300cbca	Dinoflagellata	64	0	0	0	0
bc0faec2212c942b8d7add8c68b6487a	Ochrophyta	64	0	0	0	0
9f25166c1d4128bd98ce28c444f4961d	Chlorophyta	0	64	0	0	0
0bd89be7ff008362975d744cfe779330	Ciliophora	0	40	0	0	0
614068d4a437415f3b046e7961e3ee54	Dinoflagellata	0	34	0	30	0
0d0b62554a4616386ce4fddbda8fc0f4	NA	0	0	64	0	0
f5247bb6cf494f2bc7d8681e2d75669d	Ochrophyta	0	0	0	0	64
232da0c5f8e48c8cbcbea1036d81a26b	Ciliophora	0	0	0	0	0
3aa7fa43d353b84ef65ab78dd98378e8	Haptophyta	0	23	0	40	0
0731c66ba29db529734768c42f67ec09	Dinoflagellata	0	0	0	0	63
3496876a3e59a2f95b70776ebd1d0804	Ochrophyta	0	0	0	0	63
ffa0fd417415559267a3e648e5b7ef92	Metazoa	0	0	0	0	63
941b486b5f0caa981578b6b771ce4446	Stramenopiles_X	30	0	0	32	0
26372aaf359a4914a57bc62e768ecce1	Telonemia	0	62	0	0	0
f59e30900acfd3b3cdfb888e81fa4e64	NA	0	0	28	0	34
494da0acc6b0774334705e6fb824a9f3	Chlorophyta	0	0	0	62	0
3513cd1bfc8d33f969dde3fafead4142	Dinoflagellata	0	0	0	62	0
45ec3044572a003dec3703267e0bed25	Dinoflagellata	0	0	0	62	0
00f8213f6788df680d58630617ec4e5f	Ochrophyta	0	0	26	35	0
c4814a056fce88a33867a13ceb412f97	Ochrophyta	0	0	21	27	13

185a35482380cc2ba6ea0e6c7c7c8b51	Dinoflagellata	60	0	0	0	0
de3ded5712868e4187b72703d72d8e1e	Dinoflagellata	41	16	0	0	3
9916dfb6f49022d3ab5b40921324fc3b	Dinoflagellata	0	60	0	0	0
317a12182945e72aa246e24b06e58011	Dinoflagellata	0	8	0	26	0
89af21a86cc3c1913195f4ae3496d2e1	Dinoflagellata	0	0	0	60	0
d0fa663f0dc0eef70d3f917a234776a2	Dinoflagellata	0	0	0	0	0
f32e2233dccf29f2275e471b303d1290	Metazoa	59	0	0	0	0
e4f1a1028219a045b58f7055ad7598b1	Dinoflagellata	0	28	17	14	0
4d38f40ab815b9cd99bbf30b85a580c3	Choanoflagellida	0	0	0	59	0
244559297b28b180a179df235b4d2c7b	Haptophyta	0	0	0	28	0
0e592f19b9f068905585b4ad23d162d8	Dinoflagellata	0	0	0	0	59
1631ee5420963c5876a8ab71f1939c28	Dinoflagellata	58	0	0	0	0
0522a72895d573a0fc44ac1075f8d89f	Dinoflagellata	58	0	0	0	0
27f3aa6c1120f93ac871f6b9d854fcb1	Ochrophyta	58	0	0	0	0
0e8efb2da490b95377a80e6feac9a800	Metazoa	0	58	0	0	0
a12839f64fbee38d7a6555975547767a	Dinoflagellata	0	58	0	0	0
4b5133197252e0bbaf0f66b42ccfaf74	Stramenopiles_X	0	55	3	0	0
8f29111c91314271f7367e40dd558429	Cercozoa	0	0	0	58	0
c1092e9c46cbb8dac74c4e974f7ed46a	NA	0	0	0	58	0
44c14bcec7690eb4b273a5a3bf432946	Ochrophyta	0	0	0	29	29
2fe0d81ccab06311661b1142bea3e310	Ciliophora	57	0	0	0	0
27b0e489e49b3726d3643bd440bc1c27	Dinoflagellata	16	34	7	0	0
e7c8c29c02679996828839e3e7988d0e	Ciliophora	0	57	0	0	0
91c7f0bebff04dd19879bf0ee76dff1d	Dinoflagellata	0	57	0	0	0
9d1f9539c324ad289ae25ccb456f1b3c	Ciliophora	0	0	0	0	28
7b2f7fab3a6d95f208d0acba3ea0c828	Haptophyta	0	56	0	0	0
e8cf079f188df6bee2258178ec4e401d	Fungi	0	0	0	56	0
1a312159fb9efa2aa927d8ba4bd45379	Ochrophyta	0	0	0	56	0
ba585009e647756fb0318d7749970792	Dinoflagellata	0	0	0	0	0
dcf988e8907633311be6c58e04eaf075	Dinoflagellata	0	0	0	0	0
a39e6416c5c5a5ca48e0f7a28fec0808	Dinoflagellata	0	0	0	0	0
a71cc482ac2ef32e8b1a4eff636e62a6	Dinoflagellata	55	0	0	0	0
d048f0dc7a904616e2e2816f118b57be	Ochrophyta	36	17	0	2	0
130df1b2a832f88e353536b612357192	NA	0	55	0	0	0
d51de9640ef75e3750b245ba98f634bd	Dinoflagellata	0	0	0	19	0
b17992298337ac3ab66637218efd1a19	Dinoflagellata	0	0	0	0	0
3e8485576c7213a96121eb415dcf84ce	Dinoflagellata	0	54	0	0	0
fc38fa219cff7e92c82cfeb7930a2914	Dinoflagellata	0	0	35	12	0
c1c191f112e9ab1aa3438f17c0eba9f2	NA	0	0	0	54	0
85aef17f0375093b79181297841d5cb3	NA	0	0	0	0	54
a9969a30306e10d3613006154b744157	Dinoflagellata	0	53	0	0	0
ac6895c831ee5e155c5f41ae0ea365a3	Dinoflagellata	0	0	0	53	0
d289596b2da527845fe7bc3e270b7f4e	Ochrophyta	0	0	0	0	53
21b30e8f9780d5d397336c473ac47c69	Apusomonadidae	0	0	0	0	53
0e69960a6778897d331be85461df8f14	Ciliophora	0	52	0	0	0
274d665880d5916faa533643222bb5bd	Ciliophora	0	52	0	0	0

6dfd0bf99f8d13d38c5c224cd1c6e965	Ochrophyta	0	19	0	30	3
e153d865298fcf6e4b40563ccbaeda78	Dinoflagellata	0	0	0	52	0
c24e1e97f35b304283211c85fe62cf2e	Dinoflagellata	0	0	0	51	0
ac55e2edcefa93d530e5ddda3155bb26	Dinoflagellata	50	0	0	0	0
f231bb012e641ac994987975bb579ba4	Dinoflagellata	0	50	0	0	0
1be85910ff8360e38e62d02bcd9455dd	Ciliophora	0	0	50	0	0
65f40f6d5efb7f1a957ee224c2429a6d	Ochrophyta	0	0	0	50	0
bef593113dc04794715deefa848ed5eb	Chlorophyta	0	0	0	50	0
94ab4cfb45459178c6e52d1d17c414f5	Metazoa	0	0	0	0	50
31bd9a9c97f570a4a4ac8208ab7c0f58	Ciliophora	49	0	0	0	0
5d0ed5a96fa8fed8de97829916d53918	Dinoflagellata	49	0	0	0	0
ec540d0d47c930023cb1e3fa1ea0bac7	Fungi	18	0	0	0	11
4a4a989486867b0ba974a944e624f32e	Dinoflagellata	0	49	0	0	0
98e720819c4a5c0e987411571fca54b0	Dinoflagellata	0	0	49	0	0
39d1dffd2e7dde3016de000fe6abcfa0	Dinoflagellata	0	0	35	0	0
533ca077b19a44d7abd4d0215e8c21d2	Dinoflagellata	0	0	30	0	19
084d20417b550a4bc77774da189e5445	NA	48	0	0	0	0
9296324dded811025b78837bda1266ba	Metazoa	48	0	0	0	0
934cc25faa79674eb7878d717e9161eb	Dinoflagellata	48	0	0	0	0
aae454ca54f154bc5c5b7b61915652bb	Dinoflagellata	0	48	0	0	0
4ed480d0c51eabaa2c2eee7992e0d02d	Dinoflagellata	0	0	0	48	0
f5ceed7e04fc17d5e55bbf2179d35929	Ciliophora	0	0	0	48	0
3dcb90dff701edabb863a16d5d918e38	Apicomplexa	0	0	0	0	48
ced14d9ff8e7a148023bfeb10d41a1c2	Metazoa	0	47	0	0	0
6f44bb76c72f4c07b818c8c2d14669a4	Dinoflagellata	0	47	0	0	0
7740b86bfed0c2c82e0df60a7263d8e0	Dinoflagellata	0	0	0	47	0
261359b5488a8ac48944d736c5531a76	NA	46	0	0	0	0
a793f65c5a5c9570f558ada961a93223	NA	0	46	0	0	0
824a4cbe416c0a3ed584f503558ab92c	Stramenopiles_X	0	0	0	46	0
98f81c5be31e9be8169f125a33bf073a	Dinoflagellata	0	0	0	46	0
1932183fe2b06dba56d36900fe7ace11	Haptophyta	0	0	0	46	0
c3631cad96f96916bcd7a946bc04c475	Haptophyta	0	0	0	46	0
d0b486506f6ceb1ae9e53414935f7763	Dinoflagellata	0	0	0	46	0
6bd494d49a1714486718ec850557f477	Dinoflagellata	0	0	0	46	0
d8f7b429270c48dfb262e5ba86b462b2	Haptophyta	0	0	0	16	0
2b65a8b8a05bd93cb523e680b3c5b664	Dinoflagellata	0	0	0	0	0
ca80bdd46d6a1aadf6e9cb1bb47af990	Dinoflagellata	0	0	0	0	0
0e1c5b440b155dd8549109759a139b1c	Dinoflagellata	45	0	0	0	0
0dc2eef62efb917dadcfe9e27d9dd342	Haptophyta	0	45	0	0	0
b3f2ae87137037639261a50a70f0e076	Dinoflagellata	0	0	29	7	0
b6a8adf05bdcd3e16927479376a67f9b	Metazoa	0	0	0	0	45
e5adec54a6ca452a36989964cb2fb441	Apicomplexa	0	0	0	0	45
708e86bbb7a677e0b28f909b1b821655	Ciliophora	0	0	0	0	0
eed3564a8d5cd520c430457cd1e79315	Dinoflagellata	0	0	0	0	0
d68011b2f6d72f6b4b0348d84fec1a17	Dinoflagellata	0	0	0	0	0
b0acbc0654306f74f572f077082b27f2	Choanoflagellida	0	0	0	0	0

999f4aacbd5b0f8d1a212871abf69ca6	Dinoflagellata	44	0	0	0	0
46c5c9f945110e9900779902756fbd8a	Dinoflagellata	0	44	0	0	0
c2377ee01ce4a0369b85b990a51dc697	Dinoflagellata	0	44	0	0	0
6a78a5b376b7c3ba7a63754a3afe3e42	Haptophyta	0	0	0	44	0
6808ffebd7e6f37de1de53f4d89ffeab	Dinoflagellata	0	0	0	44	0
c18b8c97f2d9ebb4a7e40804c085e0bc	Dinoflagellata	0	0	0	44	0
bd1b9801547fac0c4d3e1f25f093f129	Cryptophyta	0	0	0	44	0
44082d4da140a7e3ce8073252b01d044	Ochrophyta	0	0	0	0	44
073fd602745bdf153852f73cba424bd3	Ciliophora	0	0	0	0	44
301d1c6e18a82c84204b7d3ce62a2c1b	Stramenopiles_X	0	0	0	0	44
6e0be99e859fb804ecc3a793692bf03a	NA	43	0	0	0	0
2a38c9847dd9e53752346b7fd45e1b1b	NA	43	0	0	0	0
f7a610f082b29f133553c0f92b656790	Dinoflagellata	0	0	43	0	0
6760d7dcfbc1d47f790858993eafa3bc	Cryptophyta	0	0	0	43	0
91826c0488d6eda91475034ac5c6777c	Stramenopiles_X	0	0	0	43	0
adb0b7e92c141d317fdc1cca9f47df73	Ciliophora	0	0	0	43	0
b5d9432eafa09c9e117c7699f3665154	Dinoflagellata	0	0	0	43	0
369a9d9c5fbacc7f8a94abef415d6387	Dinoflagellata	0	0	0	43	0
24bd9a509d3d5e73e5e653589a669149	Ochrophyta	0	0	0	0	43
a7fbe02d1ec96b62ecc682717210cfd3	Dinoflagellata	0	0	0	0	43
d657fef1878fafd9f798cf6934f39f90	Ciliophora	0	0	0	0	43
ab781d6a726ce01f50dcd80b4dfeb85f	Ochrophyta	9	0	0	16	0
5f9780e898cd4942205a67cbb0e7cbd6	Katablepharidophyta	0	14	0	0	0
1aea530a8601a809702b39e646aa28c0	Dinoflagellata	0	0	0	42	0
7ad2d235c7ccd754a39d207c96e4a23c	Ciliophora	0	0	0	0	42
777c484e5c29555760e618bd743e8a52	Ciliophora	0	0	0	0	0
5b1a102d2b6c4e3ef34b422093446542	Dinoflagellata	41	0	0	0	0
24bdf536b73cad6c5673f84d211e8172	NA	41	0	0	0	0
41459f04edda70bcecedf500f9aac164	Stramenopiles_X	20	19	0	2	0
e611b6a540727483c3743d87e6170928	Dinoflagellata	0	41	0	0	0
dacb5a7ad0346685cace276f2a685a8a	Katablepharidophyta	0	41	0	0	0
40a3bd91c4b248713cfa54f2d2a434b2	Stramenopiles_X	0	41	0	0	0
1fc57be02269972b1fd2f0ac6dc8189a	Dinoflagellata	0	0	0	41	0
60b08173562542c88aadf395901735b5	Dinoflagellata	0	0	0	41	0
12e722471e8adee83f4c54530c00d82f	Dinoflagellata	0	0	0	41	0
b37e9b21ebf4a61fbfc8249f2b330a14	Haptophyta	0	0	0	41	0
ffc9ef563e124f23e2c7b506ed0bbd24	Metazoa	0	0	0	41	0
462b6a74edcdce20943fe6d1831381bc	Haptophyta	0	0	0	41	0
8aae0b978d0861dafba3410bb7e5a8b2	NA	0	0	0	0	41
2665f7da399828b5e28a2f3ee5bcbd56	Eukaryota_XX	40	0	0	0	0
f9ae1e20ba4473ea082d709e8800ca9e	Dinoflagellata	40	0	0	0	0
a493bffcab9681852d6cdf2edc4cd945	NA	40	0	0	0	0
6b4adee2aa5367f9616709c1c096bfde	Eukaryota_XX	0	40	0	0	0
e3dc360ab6dd8fc474fff98ec9eed9ac	Dinoflagellata	0	40	0	0	0
23c011c414cea0db8f41cb15ead02484	Chlorophyta	0	0	0	40	0
dae28ad0eac819f0022e942bb1651a12	Ciliophora	0	0	0	40	0

aae8ab498a7c44d45338f85bfdcf08f3	Lobosa	0	0	0	0	40
a491a9eebf5a46e3203a17655e4e8626	Ciliophora	0	0	0	0	0
0eb07bb27a1028f393a29f792803523b	Dinoflagellata	0	0	0	0	0
a312ec1f6889ee4676a254bb886cc47a	Dinoflagellata	39	0	0	0	0
1f12d52b3aa8e8879068ed67c268e18f	Dinoflagellata	39	0	0	0	0
897855303c4965d514bd12e560fd6e85	Ciliophora	0	39	0	0	0
63dfbae898a86c15e93acccca4beee0d	NA	0	39	0	0	0
b4b209543418e0f747d42962c6534eb7	Ciliophora	0	39	0	0	0
ed07ed379707ff63b97cc75998f2953f	Ciliophora	0	39	0	0	0
19ebe0503098b8fa1ce13c461aca640f	Apicomplexa	0	0	0	39	0
68682149a8ffddcbf1d1432aadaf7884	Cercozoa	0	0	0	0	39
b8f440dad1e905979abecc38f8d620b6	Ochrophyta	0	0	0	0	0
cf59d32ed177e5047730097550daae02	Dinoflagellata	0	0	0	0	0
7e4f2689d79723507642d085abe65010	Ciliophora	0	0	0	0	0
9629af07921985dff45734a5717806de	Stramenopiles_X	0	0	0	0	0
91190e466fc3166a9da53dd009eb10d7	Dinoflagellata	0	11	0	0	0
452f5e096e8700f4e6fa20307b8608f7	Ciliophora	0	0	38	0	0
ec6fd285cefe7609f03c059af3bc72b2	Dinoflagellata	0	0	0	38	0
e1ad9aeffbb84cfdf6394a6cc58af66a	Metazoa	0	0	0	38	0
463c8593f407b99b5591430be59162ae	Ochrophyta	0	0	0	0	38
0a0fac7c0acba0a2e468953c90ac8d61	Dinoflagellata	0	0	0	0	38
6ef4bf760223b5df732ad457883d9feb	Dinoflagellata	0	0	0	0	0
d1325843e80792a09bf93470612c4121	Dinoflagellata	37	0	0	0	0
0807dadf423f5e00dcd3d45388b70f1a	Dinoflagellata	0	37	0	0	0
cdb6a32a080a672428f6f53786e8b5b8	Ciliophora	0	37	0	0	0
895a03a4d83325cdee63b755c391a19e	Chlorophyta	0	23	0	14	0
d855ccf9ab0fb2e72a0f6f20fe427a3e	Choanoflagellida	0	22	0	0	0
0d189c56b66dd84c535f582835f76432	Dinoflagellata	0	0	37	0	0
81baf533cd4ce2d25a33cd18d2f1b35a	Ciliophora	0	0	0	37	0
c9e7d0349f4fe3a9ed06f4aec21f8539	Dinoflagellata	0	0	0	37	0
f133ca519968c2e1cee11034ffaa3d76	Dinoflagellata	0	0	0	37	0
9aa7dcecd4c2996fc438016619b530c0	Ciliophora	0	0	0	0	37
e2f801d715b248fa0d617b5616caed8e	Hilomonadea	0	0	0	0	37
36fa210277736382f9548cbf732f3a15	Ochrophyta	0	0	0	0	37
cfc2d3fb162e8cd01855da62ecd123fd	Dinoflagellata	0	0	0	0	0
c662e7fb38734dd2ee849c45699a1d9b	Dinoflagellata	0	0	0	0	0
666d64afb2f6caae82900b7e09021a4b	Haptophyta	0	36	0	0	0
a043c6bcf95191d818dffddd78ea01e2	Dinoflagellata	0	36	0	0	0
6033fe93a90a74c5cfd9d51f6f319fcd	Dinoflagellata	0	0	36	0	0
57b8da9ecb1f488a9dce2aed78b37ba8	Chlorophyta	0	0	0	36	0
0f0be86721ff34a2d589655ec6f7231d	Dinoflagellata	0	0	0	36	0
d22ff3174bd1daa4c6f8708c9c294332	Dinoflagellata	0	0	0	36	0
5e42dda7dbabe8954b66685e3944fd53a	Ochrophyta	0	0	0	0	36
ffaadb43fc3ab60f569fac73868b68c6	Apusomonadidae	0	0	0	0	36
fbd4b13f69380c3d015433230aa84027	Stramenopiles_X	0	0	0	0	36
c6cdc524f3d29ef10e6919cb7fa758e7	Chlorophyta	0	0	0	0	0

9470632534ba7e21f5b85e415894922b	Dinoflagellata	0	0	0	0	0
725cebc389f1e4756b5a04c993ee376a	Stramenopiles_X	20	5	0	10	0
44a069dfc00a5f2014a7b29f6bcb4923	Ciliophora	0	35	0	0	0
4ea9556a81dff70bddb914838066bd33	Haptophyta	0	35	0	0	0
4f6ea08fc2f0d0c4c03b669b44fb2260	Ciliophora	0	35	0	0	0
72153839ea9d85e726f43bf3d2812dd2	Dinoflagellata	0	19	0	0	0
a02c426bf57b2a902db8eaa6d53052b3	Ciliophora	0	0	0	35	0
5fb3e41c5654184e29b3e56b77af61b7	Metazoa	0	0	0	35	0
071d4c8f95645104b77a2f0a06e82c84	Dinoflagellata	0	0	0	22	0
b2d9c48b766d8c3c0d6fcf6596d7b80c	NA	0	0	0	0	35
7f2a129d43f62a768a925d8b1a10cf18	Ochrophyta	0	0	0	0	35
b0c1df6a648d5c5be8f26fcd7baf5cd3	NA	0	0	0	0	35
8f99e82f657228bd0d26d4bc969323d8	Dinoflagellata	0	0	0	0	0
469be80ae3d58b841030c6902efc02d8	Lobosa	34	0	0	0	0
30d9adf018a1f70b2d10b57177167faf	Ochrophyta	34	0	0	0	0
fcee830ed061f96cd886826e0d6b3426	Dinoflagellata	34	0	0	0	0
93f88f0655aab89e372175518af30592	Dinoflagellata	0	34	0	0	0
bcfff184475b46368f428f72b8bee7f4	Dinoflagellata	0	34	0	0	0
4aae5d1b51c91934d0a121422d9ca187	Dinoflagellata	0	34	0	0	0
f0143b0d57991f763a0fcedae025ca60	Chlorophyta	0	34	0	0	0
3d842c7f9bb7e3a54257a8b4e8c4e82a	Dinoflagellata	0	0	34	0	0
2f2b3baa37b26f6cbcf054c4cc5c9977	Dinoflagellata	0	0	34	0	0
fef54fc631e77f86c32a94fb828ac582	Dinoflagellata	0	0	0	34	0
221e6bdfb9bb57a1ecc62118fcc17e6e	Chlorophyta	0	0	0	34	0
1d60bc09b5221f877a1a632bc428f815	Dinoflagellata	0	0	0	34	0
5928a3dadb6880ccffe39711d19ecae0	Dinoflagellata	0	0	0	34	0
8d5a8bfd7de7828fd17328a2590225a0	Stramenopiles_X	0	0	0	34	0
4986c17163ae995eac3cc4778f2239fd	Choanoflagellida	0	0	0	0	34
498532e1b297aef5d6fa5e13a55e1d15	Ochrophyta	0	0	0	0	34
1fcb8db88f98f47dc9250711dd455413	Chlorophyta	0	0	0	0	0
304c17ea77d4b3f5c52a5f6f47cdbed0	NA	5	12	0	0	0
176706cec13523797f636b65f54123bd	Ochrophyta	0	14	0	0	0
bfae2497f5c4644b7f838b8944c7c64c	Dinoflagellata	0	0	33	0	0
e1cb487d1ac1b1144ab2c6b9effa3f8d	Radiolaria	0	0	0	33	0
9cca8bb330b75040141397bdc140ca7f	Dinoflagellata	0	0	0	33	0
a428059fd3efc2e13bb2ce8b252ad546	Dinoflagellata	0	0	0	33	0
0cc54856553c1471c52337315d245bb4	Mesomycetozoa	0	0	0	0	33
f07dc0c6e32f169e9fb95265fb10cf48	Ochrophyta	0	0	0	0	33
24311ba63b8315d6af6fd961a1e64409	NA	0	0	0	0	33
51b614ffd49f43992bc02e0038f174cc	Ciliophora	0	0	0	0	0
77cad3e8cf30c6aa03ce3185fdfa1eb3	Dinoflagellata	0	0	0	0	0
264bf120de5f286d7a0aecf21eb216ee	Ciliophora	32	0	0	0	0
b086e5ac00770d7196baadf199fadb1a	Metazoa	32	0	0	0	0
4f661ec63e6135c8e9fee603d270af46	Eukaryota_XX	32	0	0	0	0
0681a7ff33b1c39467c22b4f6f3818b7	Stramenopiles_X	0	32	0	0	0
0502c6242eaa829b30ee5e058ba81aaa	NA	0	32	0	0	0

4c08ad8aaef275ebd07f1d45bffd2914	Chlorophyta	0	32	0	0	0
756b0f2f2ee8bfc6f66568bb99c0a8e9	NA	0	32	0	0	0
61655151423407a3c814022418a048bb	NA	0	32	0	0	0
da90128c6801f9ea6d50e2f89186b83e	Dinoflagellata	0	0	0	32	0
9440ce57a2206230d4ee4ca2824e6070	Dinoflagellata	0	0	0	32	0
e9a9ddfdf7d34e1392d68156f4aab108	Eukaryota_XX	0	0	0	32	0
5274a79e79c317987b4504b0a44029d7	Dinoflagellata	0	0	0	32	0
1237876039c14bafd366e6cf98aa03f7	Dinoflagellata	0	0	0	32	0
c9f96cbc4624aac9ea7441dd132501fc	NA	0	0	0	0	32
e58b8545487d2f77cb41078c57fb7715	Dinoflagellata	31	0	0	0	0
3b11be2275177978a99cd8e8a87e2045	Dinoflagellata	0	31	0	0	0
c74fc19011a038a88dce6eba2f20c5a5	NA	0	31	0	0	0
bb6af49a4b73051e23942573d6578fb3	Dinoflagellata	0	31	0	0	0
2ff5ca64a073cdefea06f1e10c57f3cf	Dinoflagellata	0	11	0	20	0
be3e434bc4c682364ef3fc80241faa9c	Ciliophora	0	0	31	0	0
895f91299786cbdea661f963ca42a13b	Eukaryota_XX	0	0	31	0	0
275aea8a3d37cc17151d74a6309417b5	Dinoflagellata	0	0	0	31	0
b4fd1b4b3e24f0d204fc6e4684392241	Dinoflagellata	0	0	0	31	0
fac8b91f1c2bac3f04aa533cc30f8841	Cryptophyta	0	0	0	31	0
255a292c5a551491feaa660ecda08ab4	Chlorophyta	0	0	0	31	0
8adb53a32b9ab221145e487445367137	Ciliophora	0	0	0	0	31
d2a9fc945538cf5aa1d2eddd4599a27e	Eukaryota_XX	0	0	0	0	31
f7756005be6f1e2b1f536b0088d9d3ab	Dinoflagellata	0	0	0	0	0
6521bd4cd2c3b543b82e75ba8c85000b	Stramenopiles_X	0	0	0	0	0
cf9f306d3711c4513bcd0ae05968027b	Hilomonadea	30	0	0	0	0
6f72052ac1d2c21cd03e10e068024b40	Ciliophora	30	0	0	0	0
989ea1afdaeb6c09cffa437b26c9d744	Ciliophora	0	30	0	0	0
ca388498db40f49219fcc4eff92a421a	Dinoflagellata	0	30	0	0	0
71eb6c4242ed533feb4f7cd4fcded244	Metazoa	0	30	0	0	0
bea13b27b3fb94060f9bf1a7d69e51fe	Dinoflagellata	0	30	0	0	0
c83ca543f6710fac8f1bf30c17391fa7	NA	0	0	8	16	6
0d0a5037046ccb8730d5a1f56d8f441b	Ciliophora	0	0	0	30	0
a40827036a0c4693f628e404293aa1ce	Ciliophora	0	0	0	30	0
479fbafe767d3028c0392bed44df50f5	Dinoflagellata	0	0	0	30	0
2008b155777683f1ecbca57819dc2c35	Dinoflagellata	0	0	0	30	0
4e71a17c183d8c2fc95fb6a3a2f8803d	Chlorophyta	0	0	0	30	0
17c872c3d1bf25b9f5fb74dcb7b91490	Picozoa	0	0	0	30	0
272cadfd34a9999e8d70f8f90e443cbc	Metazoa	0	0	0	0	30
90a5ac8cd6bb6b13def43a52b3bac20c	Ciliophora	0	0	0	0	30
2319be09309fbb150947e6ad40f93d0d	NA	0	0	0	0	30
600572aa1f89ecafedb5f74b189ffad8	Dinoflagellata	29	0	0	0	0
46fca40f705be1706450c391c86d0e37	Radiolaria	25	0	4	0	0
3db95f6620213d38b453e6ad77dc1a0a	NA	12	0	0	0	17
836ac5a5623776cf062cc4e6d4553aeb	Stramenopiles_X	0	29	0	0	0
9783f820276a13091cbaae46b7056e6e	Haptophyta	0	29	0	0	0
f2eafd84edf02312c8de8522194c6973	Dinoflagellata	0	29	0	0	0

7fe03cbd39d699a4a35b8fe7fc5fafe7	Chlorophyta	0	29	0	0	0
8180cd36dc5cb832204775acf9625a02	Ochrophyta	0	13	16	0	0
e6f1fb7add8f786c0ce2a28588282e8e	Dinoflagellata	0	0	29	0	0
2491abd9880291095421d68154964e04	Dinoflagellata	0	0	29	0	0
4c392265f6e8c87bd4880dc37b6cb1a5	Dinoflagellata	0	0	0	29	0
2e90a6578ddd9e48fb4bb725c8a68b2e	Dinoflagellata	0	0	0	29	0
07b965ee90543c9c9ee7a48e05dc2dbd	Dinoflagellata	0	0	0	29	0
c0ce0cfe8543bff976f2b7a2febe8e13	Dinoflagellata	0	0	0	29	0
9e0d3fe1e2961b789069d41923613214	Choanoflagellida	0	0	0	29	0
5d5af9437099788261f40f226b3eccc0	Fungi	0	0	0	29	0
01c0c8a78b257a871f7198583fa64943	Dinoflagellata	0	0	0	29	0
6028eb336e3e5ffe887dc0b772e3d9de	Dinoflagellata	0	0	0	29	0
7b13bce7323a650fb19f6971d2e1a1f3	Dinoflagellata	0	0	0	29	0
5ca3e270aed65eae9418eab4314ca1c6	Eukaryota_XX	0	0	0	0	29
9d29bdb8b077ef3e6b9a6b295404562c	Metazoa	0	0	0	0	29
cf45a20b731a6f8afdd1a57cff796479	Dinoflagellata	0	0	0	0	29
df8ce5dabda34f9b442f59e36439532b	Haptophyta	0	0	0	0	0
8db634bb958598f0abfcd24e81ff5c38	Picozoa	0	0	0	0	0
ba7c597502e6d8dcd289789b6eef02c5	Ciliophora	0	0	0	0	0
1508ccf7ab0885ed99b9a0d043570d90	Dinoflagellata	0	0	0	0	0
80554a6de8bfcc1ca6e7d11ddcfb9b12	Dinoflagellata	0	0	0	0	0
5f8d9ccdc8bc52dce463e00d75c8ed8d	Dinoflagellata	28	0	0	0	0
24e1da327f6371ca2a7eea8d5132cd9e	Stramenopiles_X	28	0	0	0	0
dc907e110ca9a547cea21d4d491dda27	Chlorophyta	0	28	0	0	0
216a85c33dd585dee39452018ce38800	NA	0	28	0	0	0
42fea8b7368f95f217eaa226777e94c1	Dinoflagellata	0	28	0	0	0
4656c27e3a54775e1cd8fd18a81181af	Dinoflagellata	0	0	28	0	0
d2afd5559df75f5e8e9f2b6328e68233	Dinoflagellata	0	0	0	28	0
006b6477d92a681d61d78bcf79788283	Chlorophyta	0	0	0	28	0
5b9d532d519d9b581f2b431c1555061c	Dinoflagellata	0	0	0	28	0
93561ab79b665257c2dc27b91fc10519	Stramenopiles_X	0	0	0	0	28
fd565a0e3969eeb135e8da4bd50efb8f	Dinoflagellata	0	0	0	0	0
3e4f323a862e5cac53f2d4fd934b0684	NA	27	0	0	0	0
eb488ebd279255775375401 9b42c08f2	Ciliophora	27	0	0	0	0
63571a993258de066eeb6c0cf5e2af82	Dinoflagellata	27	0	0	0	0
c2b99dfc75dd77db9313116482e2f391	Metazoa	27	0	0	0	0
e2c3b88ce5782ac163b0cec3ea055bc7	Dinoflagellata	27	0	0	0	0
7c24910472c32e1ff6532a35c416b089	Ochrophyta	0	27	0	0	0
6968e57d86e25db41a408dc9b6918b2e	Chlorophyta	0	27	0	0	0
d77f6cb9b5183d8879d96bdc174d571b	NA	0	27	0	0	0
6361e4d3afdcd8b7ecca803c5faf0683	Stramenopiles_X	0	27	0	0	0
7ed0590de37c792dd97935b198d3ac1e	Dinoflagellata	0	27	0	0	0
cefb1c0dc5b931af35d6f7aa74d43cab	Apusomonadidae	0	0	27	0	0
ab7e862c7766bad474ae1425f5efdfd2	Dinoflagellata	0	0	27	0	0
2e3e87f586efa3d8c839b4386f39bb75	Ciliophora	0	0	0	27	0
1019062971d5b0fbb9df57c42c9abe25	Dinoflagellata	0	0	0	27	0

c0ea9c4633cb18ae83c1d26a1dc43f59	Dinoflagellata	0	0	0	18	0
27dc46530a7f05c1beebe660b5ccf279	Lobosa	0	0	0	0	27
702368b48d42e947065a0a0d1b9e2b92	NA	26	0	0	0	0
11cd84fedbd468e90f326c4cc4c3b985	Chlorophyta	0	26	0	0	0
a8866301e2cdf65335786427b644e734	Choanoflagellida	0	26	0	0	0
efceadae049b1ccbb520d273aba6e89e	Dinoflagellata	0	26	0	0	0
79fe1e94685551a4bcd33ea77455d1d5	Dinoflagellata	0	26	0	0	0
fd5ae246f664d8a76c64b564ced6ee76	Radiolaria	0	26	0	0	0
e97ea00c573e128255cdd3c704683b28	Chlorophyta	0	26	0	0	0
e6d102aa157080c786f2ab94d78d0cf9	Picozoa	0	26	0	0	0
59e31010fa1c881566ec0d730be13e20	Ochrophyta	0	26	0	0	0
d99d4fb8154b8b79cb4a6335b8bbd069	Katablepharidophyta	0	26	0	0	0
38b6ef7ace2935041c0a8406d275ae1d	Lobosa	0	0	26	0	0
157f663f6d161063fec8f331bfc193bd	Dinoflagellata	0	0	0	26	0
da60ae2f665840c5cbe908e94f503f97	Cryptophyta	0	0	0	26	0
190d1c3f6294b10c58696ed2f957bb24	Dinoflagellata	0	0	0	26	0
017afbce49af275162f150b5e3a3b102	Cercozoa	0	0	0	26	0
d7e46d4ff4a541f8f3a8d04e9434e740	Dinoflagellata	0	0	0	26	0
ad3015257771094374a6f8f2bae42205	Dinoflagellata	0	0	0	26	0
23d8816f442eccd0ce1e41cc90719194	Dinoflagellata	0	0	0	26	0
d99e72d55a3a57e05dceedcaa91b535b	Stramenopiles_X	0	0	0	0	26
808417ad9960a3638b30933e21912e0a	Apicomplexa	0	0	0	0	26
f27b196b312426ac09f5136f137965fa	Metazoa	0	0	0	0	26
b01a3fb872502d8b5aa16150408bcf4f	Ochrophyta	0	0	0	0	26
680497ff5dd48c3f878f44625293ccc0	Cercozoa	0	0	0	0	26
a4799a6370088f38d0a004c962dfa1c8	Fungi	0	0	0	0	26
6d6b63ce126d9f45d64faf2a4888471d	Dinoflagellata	0	0	0	0	0
a830ab9ef1ab43cfc7b3d0f6d1d9716a	NA	0	0	0	0	0
4d4045868af3ca390306ba26387600ca	Dinoflagellata	25	0	0	0	0
38183b9b2092a24636b52912a27d136f	Dinoflagellata	4	0	21	0	0
03fe2dae5cd68e85106a5555f501f88b	Dinoflagellata	0	25	0	0	0
56874481277779441f3474722294d17e	Chlorophyta	0	25	0	0	0
6600f1b85502bfef54d286ff616405cd	Chlorophyta	0	25	0	0	0
dd778817acee047b45bb6622a3be7914	Ciliophora	0	25	0	0	0
f59e8b8b5e2d14fe359a9182278b6140	Eukaryota_XX	0	25	0	0	0
2d1a0df66b53e67fda9de6d7a99fcaaf	Eukaryota_XX	0	25	0	0	0
898bbe1ccb04a13e5d3df6e7665be3ed	Eukaryota_XX	0	25	0	0	0
dea94c1900282290a331a9d463a246e3	Choanoflagellida	0	13	0	0	0
eabf0630aab19349e412792aa4e20b3e	Dinoflagellata	0	8	0	17	0
1dc6556428bfae2d056608fe020c348d	Dinoflagellata	0	0	0	25	0
9b88043961a16745009bbfaed9cd0aee	Dinoflagellata	0	0	0	25	0
8141ba052fa45ae73531891cbc83c915	Dinoflagellata	0	0	0	25	0
6b853907c64696098298fda0724bbc88	Dinoflagellata	0	0	0	25	0
1c11edbbcf7d59ab124f79c912f41e6c	Dinoflagellata	0	0	0	25	0
dd7b862c22649f0e1f3d8d622469ebff	Chlorophyta	0	0	0	9	0
63188ea650b72632329741725f7ac0f5	NA	0	0	0	0	25

8520201d28785606e17e99ac37147f6a	Lobosa	0	0	0	0	25
9df4ad728eb1df786a6c00b7edb2d545	NA	0	0	0	0	25
edccc2cf22468f88bc87f0530e54d06c	Cryptophyta	0	0	0	0	0
53d6ea05803c80f04603433ae932ccfc	Dinoflagellata	24	0	0	0	0
31e6ff832d3474edacc1672077ba1e6d	Ochrophyta	4	0	2	1	0
76d9aae3745fc402daa5808eb86ef746	Ciliophora	0	24	0	0	0
9b4463b19dea41ab18060746babcb2fc	Ciliophora	0	24	0	0	0
52329dbe0011caad8a52da1119b0c58a	Haptophyta	0	24	0	0	0
6c16bb5b2d686de17a46e22a1990c9f8	Telonemia	0	24	0	0	0
4e5550840eda460fc8290317125d041d	Chlorophyta	0	15	0	0	0
7f639ea8242019442b29015cf7c78e04	Eukaryota_XX	0	0	0	24	0
ebae8caa0bd867463ddb6be79384d823	Dinoflagellata	0	0	0	24	0
8bc0fee24e96dc2411c474aa84065a8a	Dinoflagellata	0	0	0	24	0
06379400decefcaf643b48e08a3559be	Haptophyta	0	0	0	24	0
32edb6257dcf9aaa71cd1e46fae54958	Eukaryota_XX	0	0	0	0	24
edc789ca72c2bfd77232292cea50718c	Ciliophora	0	0	0	0	24
242383cd00e2c405320f15c7b3d01bf7	Stramenopiles_X	0	0	0	0	24
2e31682630bedaf4eaec2a6b753dd459	Lobosa	0	0	0	0	24
4247106fdb91dbfc9899c80f7cc0199f	Apusomonadidae	0	0	0	0	24
cd34d774588f528fc971a44f15578133	Stramenopiles_X	0	0	0	0	0
da597fb80fe2825a2a212af79fd778cb	Dinoflagellata	0	0	0	0	0
3e89338263f6deb7dd9de4fa88d8c3ea	NA	23	0	0	0	0
e975e8376953e55d5e531bb838525c62	Dinoflagellata	23	0	0	0	0
1aef64d4524e9f0d772eeb57fee1b021	NA	23	0	0	0	0
a49dca498434edf770d7783b9c41e065	Chlorophyta	0	23	0	0	0
fd8b08915af49134005bbcb7db3879a0	Haptophyta	0	23	0	0	0
60b607cb7c0f7aa0752c2986bdb6e6c7	Ciliophora	0	23	0	0	0
752292ef5418e2544bc1b5680473fb60	Dinoflagellata	0	23	0	0	0
1e2d94b5f0bb1c016c8444144c77e0ab	Dinoflagellata	0	23	0	0	0
e05687c603784f60d7aa717f26f27aab	Ciliophora	0	23	0	0	0
fc7e6609513ff426365e68b1818c0ab9	Ciliophora	0	23	0	0	0
d89df3c25e224012131d31d55fcf0b8a	Dinoflagellata	0	23	0	0	0
1505feafe41e9f699dc93863a5ca4ea1	Ciliophora	0	23	0	0	0
172325c9b5f200c45c616d3b900b7e60	Dinoflagellata	0	0	0	23	0
f65c8443c2bfb4e1406eab4adfd41b99	Cryptophyta	0	0	0	23	0
a970ab8b7a352f8a3887b5f043cc0138	Chlorophyta	0	0	0	23	0
b86de5fe96b9298754693d843ad1612f	Dinoflagellata	0	0	0	23	0
2c6912e092b8e112b9ba198d6bc81312	Dinoflagellata	0	0	0	23	0
30fe5c5162be961af6caa550254df7bf	Dinoflagellata	0	0	0	23	0
dae4c5ec8fe9faab564aac3966b4b92d	Chlorophyta	0	0	0	23	0
7618d9f10f2874e7c82675617d2700b4	Dinoflagellata	0	0	0	23	0
47687c8dc4d1b2504613aba972c8ac76	Ciliophora	0	0	0	23	0
09194a32e386493bdc69e53a5b8d667b	Dinoflagellata	0	0	0	23	0
9a071838c6a1fffdcfca3a1068e5ce6c	Dinoflagellata	0	0	0	23	0
ddf52333404a5c84191e07d396a971ad	NA	0	0	0	23	0
c0b830cea625299ae90463243c5ba730	Dinoflagellata	0	0	0	23	0

1435386f4c69ee4cd47a9d1baf6561ca	Dinoflagellata	0	0	0	23	0
cc1f334a087d1059ecd189c9aae987d9	Dinoflagellata	0	0	0	23	0
92f973daa0f507e8768439a9e0f5335c	Dinoflagellata	0	0	0	23	0
b55b52508c59f1794040e10a485816ff	Dinoflagellata	0	0	0	23	0
a9ded133fea3a899416bfa09d59a4f85	Stramenopiles_X	0	0	0	0	23
4e801d860926c494741fa88c2393dceb	Metazoa	0	0	0	0	23
1b7fd72db213770a4c74f78bb8b673ed	Metazoa	0	0	0	0	23
5f86c54241690c69c4c9806cf82310fd	Ciliophora	0	0	0	0	0
7d6054d4ee260db29a35be443b5445fe	Chlorophyta	0	0	0	0	0
10414583536975dadcceef453ac09707	NA	22	0	0	0	0
4ed7318cca8a625d34435c8bba68b812	Dinoflagellata	22	0	0	0	0
059a9b8fb49be8ede29485b90a28ca7e	Eukaryota_XX	22	0	0	0	0
ed0a4853d8f046bf9ef273a5c8829a26	Eukaryota_XX	22	0	0	0	0
e1b53433208cd5edd758ce4ebe0cb120	Dinoflagellata	7	0	0	0	15
16d2ba1a412e877125058f573aefdf79	Cryptophyta	0	22	0	0	0
d0f994c57c1c5383c298a535310ec792	Dinoflagellata	0	22	0	0	0
d68bacd5ed72b1e8e57105d9fd29d058	Dinoflagellata	0	0	0	22	0
296c85a1a173f628cfe6e518a6dc5cdb	Dinoflagellata	0	0	0	22	0
7e9709be83f6fe890e254d60e2730c0a	Dinoflagellata	0	0	0	22	0
dc7b4bc9c3c327485dc84821be1ce79b	Apusomonadidae	0	0	0	0	22
94f228d82277f37b0d81bfb0ed333eff	Eukaryota_XX	0	0	0	0	22
5920c7a2289c9e0cc1af34e7d02e8c2f	Dinoflagellata	0	0	0	0	22
5637e83c58f6daaad77de8b0b2ddeaf1	Dinoflagellata	0	0	0	0	22
3c51267278e1669bd00a6c7bbc46de16	NA	0	0	0	0	22
6ee92de7c3a86be7e1d89bcd697ea3c9	Ciliophora	0	0	0	0	0
ccca404f883f9b0489c9001f8c27c11a	Apusomonadidae	21	0	0	0	0
f02d5f82a3346c453fb643fd40008f81	Metazoa	21	0	0	0	0
00624a5a5edda165f6701d592267803f	Eukaryota_XX	21	0	0	0	0
6ba4948582caa9c4d51317f3e67de4f1	NA	21	0	0	0	0
70dd22556335b2b46e9baedd97244895	Ochrophyta	21	0	0	0	0
7a29733530f3211f6fe061f8b47dd3ea	Metazoa	21	0	0	0	0
a16d820ff6b2749ec3bbe8ac900cba33	NA	21	0	0	0	0
fabcf04d291d1b450806b06ce10034a0	Apusomonadidae	12	0	9	0	0
9201e70c8e30a227e6efbf6cec8ad795	Eukaryota_XX	0	21	0	0	0
1ae9537156d2fe1bfff5ff8a661c9957	Dinoflagellata	0	21	0	0	0
3a172aea87820b3ccc1fdba20296eecb	NA	0	0	21	0	0
487915f1652d7e014463bd892a4f32f1	Dinoflagellata	0	0	21	0	0
2dfc8ccdb5f5ed0a9033feee2ba0a4bc	Dinoflagellata	0	0	13	0	0
55803e3e042d2ee5c823c2f4cbdced15	Ciliophora	0	0	0	21	0
bf93ab6d1d8c555040521f87883f03dd	Dinoflagellata	0	0	0	21	0
305fbbedfe3a275cac7a9a9738abcb33	Cryptophyta	0	0	0	21	0
7b0655444028d1d5cacc8afb30e9716d	Dinoflagellata	0	0	0	21	0
e40f04538ddb9a2f1c54d7d3b15be35a	Dinoflagellata	0	0	0	21	0
460c10b5d0cef42af76b21699fa99056	Choanoflagellida	0	0	0	21	0
2060c0e0cc8176ec343adc0e2e9e1da1	Katablepharidophyta	0	0	0	21	0
9c5bfe36936c8435dfe542edf1ed933b	Cryptophyta	0	0	0	21	0

9f3dbc0cec09be60635870f57c689b9c	Dinoflagellata	0	0	0	21	0
8044a4ad5d3748327dcd3946fff547b0	Ciliophora	0	0	0	21	0
b013499e6a2e63a7a9a73faa24aad20a	Fungi	0	0	0	0	21
1f2af4aca86478f109fdb1baea807228	Ochrophyta	0	0	0	0	21
ae44d3b57569093b7da3faf67601f68b	Ciliophora	0	0	0	0	21
641f2a8053a78793ff1782c4512f85c0	Eukaryota_XX	0	0	0	0	21
56f26e50ede58858cdc2e510d9d3053e	Cercozoa	0	0	0	0	21
5d7e4136f08d23147f92d3928898dd4b	Fungi	0	0	0	0	21
4505fc506afda2fd50a35a5bfc9243ef	Fungi	0	0	0	0	21
c395be58e90d741a87ec003f8878131c	Dinoflagellata	0	0	0	0	0
1c58716ee6fb0705357fe4e4c61e83f9	NA	0	0	0	0	0
18dbbf556b677eadfbf8f840787afe53	Dinoflagellata	0	0	0	0	0
fc4763676558ce226c57515fdc950246	Dinoflagellata	0	0	0	0	0
e4ce22c8ba5de5355cfd2e8cdddaa72a	Eukaryota_XX	20	0	0	0	0
08408ca1128f708993dfa7947187c4dd	Apusomonadidae	20	0	0	0	0
4a3ea673830b774b705dfda84ac0abd6	NA	0	20	0	0	0
0681b9572d4043de9d97f9e4bc6dad22	Chlorophyta	0	20	0	0	0
43e647fffb82648c813e6e68e44d01fc	Dinoflagellata	0	20	0	0	0
9b3171ad33d2bcfe5ea0c270c53b386b	Dinoflagellata	0	20	0	0	0
61866f4eb26b219cd7650f99aeeaf0c6	NA	0	20	0	0	0
1a25e688f45eba91016a2d409d2b1f5e	Dinoflagellata	0	20	0	0	0
088515be06ec341ac5f12f074ee42932	Eukaryota_XX	0	0	20	0	0
728c0dd811dd7bbf3a86e0184fc036f4	Dinoflagellata	0	0	20	0	0
f3b1b1b89bee2f2bbfff47eafe2a70ef	Picozoa	0	0	0	20	0
510f7b272c73c60344ef666387c61cbc	Cryptophyta	0	0	0	20	0
a7133a9f2e4b756e3874a21405814c46	Metazoa	0	0	0	20	0
e87734cb1b77cab4ee4f46e0ee57fab3	Dinoflagellata	0	0	0	20	0
eab1e0dd9113b0aeb75ecaa17127dfb9	Dinoflagellata	0	0	0	20	0
bb45d2ec053918bbbc50f5f2e8f0ecd5	Ochrophyta	0	0	0	20	0
33e705b5340fe359fc1856631dd8da75	Chlorophyta	0	0	0	20	0
7b5a83bba328dcc6abc1944efe3581cf	Dinoflagellata	0	0	0	20	0
e4ece672c42d221b08d66e12eaf0c73f	Stramenopiles_X	0	0	0	0	20
4e95245cb640cae22e9bd76b99d7c994	Ciliophora	0	0	0	0	20
f714d5d434d6078915a3e57c5ce11dd4	NA	0	0	0	0	20
cea3633534d88bdb770298cdf2856e9a	Lobosa	0	0	0	0	20
ba729d5583eb50cd9bf860751d32099e	NA	0	0	0	0	20
c643b90791dd4b1099bf26640c085cbd	Katablepharidophyta	0	0	0	0	0
13eab55a7ed0bc274bdedf2ab27a0bbf	Dinoflagellata	19	0	0	0	0
4df28facdd385ad9ff74bee54c42e4ef	Dinoflagellata	19	0	0	0	0
98a5454d274db9eb167249536fd28ec7	Ciliophora	19	0	0	0	0
227a8d9f440f9c067b3eea19838a7363	Ochrophyta	13	0	0	6	0
7da08517775862f207507ab7e90fb4c4	Haptophyta	0	19	0	0	0
8032e4b07a2158cd64b0a175f2ef4bc1	Haptophyta	0	19	0	0	0
977ae9afed760088c8bd6f68c57cd4e6	NA	0	19	0	0	0
a20d205e20b9957d1790e84a6e9e8234	Dinoflagellata	0	19	0	0	0
b356e63b2826b96a79474629d89a52e3	Chlorophyta	0	16	0	3	0

42b9bfebb2f5891e585e32c103b10d9b	Chlorophyta	0	15	0	0	0
8f789395ea75f2898193d86ba3dc5586	Dinoflagellata	0	0	19	0	0
1073d0417a9a541eed501caead6decc0	Stramenopiles_X	0	0	19	0	0
c0ecabfc94ff0881eb086cf2fb74088d	Dinoflagellata	0	0	19	0	0
f21cbba2ad9ffd2707a1377802967db6	Ciliophora	0	0	0	19	0
0eb6b179e2c0d3931be9fde2fb05808b	Dinoflagellata	0	0	0	19	0
6df50aa13b6b899da2f51e88b9ef8917	Eukaryota_XX	0	0	0	19	0
d697c4bbe38420d030bb96feb863888d	Dinoflagellata	0	0	0	19	0
c3a86f296805c667bc69f45b7b387d1f	Dinoflagellata	0	0	0	19	0
78e3d016f1be6edccd1c8e9686f88513	Chlorophyta	0	0	0	19	0
6c4c6980891cf143da45ccde2f1a49e4	Dinoflagellata	0	0	0	19	0
2a9b90cc88c2f9e8687494dc8c3ab8d6	Choanoflagellida	0	0	0	19	0
c9cff5be383af91e06f097b358b22e6d	NA	0	0	0	0	19
24345d28b8e7d814b8f4bb2cdc2d6969	Ochrophyta	0	0	0	0	19
8d71059dfd2a43848021b48074053350	Ciliophora	0	0	0	0	19
aeeac7ed6ad66a9d9abc02bd4b2f3ffb	NA	0	0	0	0	19
29b909a7ffbf5ddf7c1a8304ba0aae5d	Ciliophora	0	0	0	0	0
1db47dd14267cd660187f5e573724119	Dinoflagellata	0	0	0	0	0
f6850cbf6523618bfda459f1586515f4	Radiolaria	0	0	0	0	0
7667afcf7b5bec4271d3738daab1788f	NA	18	0	0	0	0
bc9ecbe7baa92d8dd1dd72818fdf7ee3	Metazoa	18	0	0	0	0
e2f0f2045c8e651d33eed889977a1fb2	Eukaryota_XX	18	0	0	0	0
2a2e6564d44cc445046cbe8bca4c30b6	Choanoflagellida	0	18	0	0	0
2ddf1801a0a0e08f37f3ff8b19924e44	Dinoflagellata	0	18	0	0	0
e445735a2ac340735fd25a65443a12a2	Ciliophora	0	18	0	0	0
c9731e47d54ab85333f41888919671fc	Metazoa	0	18	0	0	0
5f87c7078a8743b9681f2bc4a934e77d	Dinoflagellata	0	18	0	0	0
29514808fd05d5be79b1a656a3c6455d	Dinoflagellata	0	0	18	0	0
07ce4d2d92d99b69768a8da3115b1a84	Dinoflagellata	0	0	18	0	0
7bf06d409384035c92dc008466155ef7	NA	0	0	18	0	0
193654140f6f706f1ca242d159951ad3	Dinoflagellata	0	0	18	0	0
95cf5df684a490a1ac5bd9917903a700	Dinoflagellata	0	0	18	0	0
0b6b7e1e88b231c8d59d8008d07e5c67	NA	0	0	18	0	0
890b9515fd4256b320db3f156aa4f7d1	Apusomonadidae	0	0	18	0	0
17b7dae0505d0301bf0968349a0490e9	Ciliophora	0	0	18	0	0
f16a0e6f65ad41fd8352019d07273517	Haptophyta	0	0	0	18	0
df7ff4d2ef30ed81b9ac4214c323c8c4	Dinoflagellata	0	0	0	18	0
7fbccebf8fae07b42317acd8b5daaca8	NA	0	0	0	18	0
ba9538f9a0f59225989f3ae5b3cd73ca	Dinoflagellata	0	0	0	18	0
dd58500d9fb4460b77cd61962a4291d1	Dinoflagellata	0	0	0	18	0
eca8d62191b564e541b06082224c56d0	Haptophyta	0	0	0	18	0
74c2a69068d6836b2fcf62a6e60af122	Dinoflagellata	0	0	0	18	0
ccf4acda95cb3789519dee2999d02c82	Dinoflagellata	0	0	0	10	8
b972695abd12c2c09a81ea4b61c444e8	Ochrophyta	0	0	0	0	18
e2170f9c3443a6b3099e548289f066c6	Ochrophyta	0	0	0	0	18
eea7710314cf172a2d3d4af9d66cdf6b	NA	0	0	0	0	18

af789a429b59ad487303dd44a24c0209	NA	0	0	0	0	18
884441a460e10e7f59708814f1679f96	Ochrophyta	0	0	0	0	18
b9dc660b90e59a44bb9ebd66bc41a4ab	NA	0	0	0	0	18
6a7385b8228ddfb5737f81364a5e7f6f	NA	0	0	0	0	18
0ae724231f0d53e2a42c51ded32f22c0	Picozoa	0	0	0	0	0
1d396fc1cf9942da83f057668a7a5d84	Eukaryota_XX	0	0	0	0	0
0cc482c7b1b4db5c36ddc44e845bbb89	Dinoflagellata	0	0	0	0	0
1fc3e4769db9689eee24525af01a6db6	Ciliophora	0	0	0	0	0
755d3464286c3a884799e28091b0c244	Dinoflagellata	0	0	0	0	0
7e331229ae87b14c6c5bed49f1387a62	Dinoflagellata	0	0	0	0	0
245a347679c2a5cc934b1fa4c8d7d476	Dinoflagellata	0	0	0	0	0
0b0bde35b9ebac9dd423d2e485c0886d	Dinoflagellata	17	0	0	0	0
1fcdadee2c54ca1d8852754072d7e485	Ciliophora	17	0	0	0	0
0660d65569b77a51737e187a96fe0816	Ochrophyta	17	0	0	0	0
a147ce9e58d1650cd8be6374e0cdfff3	Chlorophyta	0	17	0	0	0
c6aa70d68b7c7690c239e5a7ae979518	Dinoflagellata	0	17	0	0	0
9b0c9ae7da8fadafafb4bc97e5ae276f	Dinoflagellata	0	17	0	0	0
d4117d43c3bd1fa6f4ee1859f13dcce8	Chlorophyta	0	17	0	0	0
9a31c6a8a9866a24ca1ad16e68929fe3	Dinoflagellata	0	0	17	0	0
d9b5ac2ee8240d91740d6dfa37b801bb	Dinoflagellata	0	0	17	0	0
8cb427fb9adfe0c5543c853bf769139b	Eukaryota_XX	0	0	8	0	9
4fd0f5f2f4da02e04b3eecc390a6b526	NA	0	0	0	17	0
b7dfecc29ccdf72b56f18f650e7b2786	Dinoflagellata	0	0	0	17	0
ae0da2fdbfc18b19b1adf5fc83593fd7	Dinoflagellata	0	0	0	17	0
37afc47971750d9f5fa058767554f78d	Dinoflagellata	0	0	0	17	0
1bf7a95ebf588afea17aee89684b752e	Dinoflagellata	0	0	0	17	0
3cbd2ad505a4292df73a0d0eeed83b98	Haptophyta	0	0	0	17	0
4527aa70197f4b3d13699fdbbe61e46e	Metazoa	0	0	0	17	0
3c1ac44affa26f14a48e20fffd54df5e	Ochrophyta	0	0	0	17	0
b0ba65fe20fc769a096d236dd0ab2b41	Ciliophora	0	0	0	17	0
cff9b1427fbfbcfee9789fec3b26868c	Dinoflagellata	0	0	0	17	0
285b56354a5651986cfe6bc0829e525a	Dinoflagellata	0	0	0	17	0
3ddad4fda006f9cf0f32d3fcdf6f2b16	Eukaryota_XX	0	0	0	0	17
c009f2e16ec84a84e76f5b7fac6bf97c	Eukaryota_XX	0	0	0	0	17
ee498dd90079096e100c100b25898b74	Ochrophyta	0	0	0	0	17
31073e7339e95123ed982c3394adabe6	Fungi	0	0	0	0	17
9bb2a1ca924cd04649d95cc409db4909	NA	0	0	0	0	17
722086e39121e0544436148d676a8ac0	Metazoa	0	0	0	0	17
560de519f5e53b02fcd17cd2b4f2313f	Ochrophyta	0	0	0	0	17
7070113d27f07fc8908ec16d03110a7f	NA	0	0	0	0	17
1c1d71fd11cc02d8578e18e052b355a2	Metazoa	0	0	0	0	17
f8c9d0ba9f6601a6c937f1c3b9257b9b	NA	0	0	0	0	17
65bc7455b4a12498f106abe1566debb2	Metazoa	0	0	0	0	17
56f6471adb0b94b6d6cb3fbd71e25a5f	Ciliophora	0	0	0	0	0
bf6c64ab852fe2417d9428cbc19087e2	Stramenopiles_X	16	0	0	0	0
53ec670cc145d68db14a47f6d8b6c7a7	Dinoflagellata	0	16	0	0	0

ba93f31da109c609ab84d3968fa574a5	Dinoflagellata	0	16	0	0	0
6cb86c2290dfc3eb1b67049e9c031726	Chlorophyta	0	16	0	0	0
0283617839efd10e7e96b138288c2db7	Chlorophyta	0	16	0	0	0
9c41f5391f9bc38e2d136a7093423dc9	Eukaryota_XX	0	16	0	0	0
8b9289b8c6257fd3c69949012776e3e1	Chlorophyta	0	16	0	0	0
242d150aab2c777672d0af8de859b15b	NA	0	0	16	0	0
d73a94091170c629f3a6cc2c8adabc8b	Eukaryota_XX	0	0	16	0	0
46fa6facac2f27038c0112b89b4d8a07	Chlorophyta	0	0	0	16	0
782bf8707b410be4d9f2b426c43726d7	Dinoflagellata	0	0	0	16	0
5397c132887fff9fa63a0bba72efb7b5	Dinoflagellata	0	0	0	16	0
d6b52d65656b3a68591e38c0afb6e6f0	Chlorophyta	0	0	0	16	0
2053ca185350ba66968d7febc6d997e5	NA	0	0	0	16	0
30e2bb61570603d40f3292a7701517d9	NA	0	0	0	16	0
b099a0090a79100af620025cb562d72a	Dinoflagellata	0	0	0	16	0
79302b154002b3c0dacd9564f9a2135c	Chlorophyta	0	0	0	16	0
91e6b3236bffca14e6b17ae3c2821076	Dinoflagellata	0	0	0	16	0
7832a8988327e158e6b2336632e21e62	Dinoflagellata	0	0	0	16	0
02949191c919aaf3a8d1143670cfd287	Dinoflagellata	0	0	0	16	0
d952602c2ba3bc230203bd18ea0c23a0	Dinoflagellata	0	0	0	16	0
3f31c1b1257a1ece2855de2ef783ceae	Ciliophora	0	0	0	16	0
5ef188acca10d8ca716370b56c710e0e	Stramenopiles_X	0	0	0	0	16
c710ddb83f077f7f727b1a938ae5cf0b	NA	0	0	0	0	16
9a2aa8576104a9c1a3cb008ec4147b7d	Apicomplexa	0	0	0	0	16
07db2db29e0030c25b68cd4d93843273	NA	0	0	0	0	16
913cf0050f872c0672885ff8e57003c8	NA	0	0	0	0	16
3c1a21e81b8de4e0cad4abdee06a9194	Ochrophyta	0	0	0	0	16
70bf5258d5ffbe344b3f6b7860bf822c	Cryptophyta	0	0	0	0	16
322c1dea1311028fdbe07bc986349f33	Ochrophyta	0	0	0	0	16
ae8c49863f95b7cbbe074933956bc59d	Ciliophora	0	0	0	0	16
2c622a28b9568327e19f0ec99c46130d	NA	0	0	0	0	16
be10f4567d5e8b1f0deaed40b951adca	Chlorophyta	0	0	0	0	0
f6ce77d98cf85c5b7e0efe211cfe3e6e	Dinoflagellata	0	0	0	0	0
cce69fa62c2fd9123890a1e34251344a	Choanoflagellida	0	0	0	0	0
843d7d5d1acf77169b46a3f084a5983a	Dinoflagellata	0	0	0	0	0
9c30f2e40a0df7eba7e405b1286fb542	Haptophyta	0	0	0	0	0
bdaba6a1a29ad30d16b468f63ce1607c	Dinoflagellata	15	0	0	0	0
f8fe835bc4c9a0441c2ac823cb7c3438	Metazoa	15	0	0	0	0
0edf7c30dd92690ee512fc2493f38b60	Choanoflagellida	6	9	0	0	0
a3f8126a5bffd5f39f407862e18e928f	Dinoflagellata	0	15	0	0	0
a25295b86c457e3795000720c663ab00	Chlorophyta	0	15	0	0	0
1e02f3229488c5643188ecb484e1b699	Ciliophora	0	15	0	0	0
85a73f34f5f112c4e12e045d665aec62	NA	0	15	0	0	0
137af7a582b1e98bcd562c5bdfe8dccb	Dinoflagellata	0	15	0	0	0
a37edcd404d46686845fe2dd895998a7	Metazoa	0	15	0	0	0
e3f8d67be31b2f02b63e0f74e396e940	Dinoflagellata	0	15	0	0	0
2c32ba09f2a96b0a0c6483df2cba4f55	Metazoa	0	15	0	0	0

b6a3f63fecf3d2bf560992825f1f8dcd	Chlorophyta	0	15	0	0	0
9ee3483e635a5a3967772ee4fc14a690	Lobosa	0	0	15	0	0
2c19a89236381ca2f4402f629b932496	Dinoflagellata	0	0	15	0	0
dea0fdaa0ca808786c35de8feed00fc8	Eukaryota_XX	0	0	8	0	7
8b6dd8aacae2a125c73a9f7ecb8de3e4	Dinoflagellata	0	0	0	15	0
121e6bf86f089c62b0f0457c7d45221d	Ciliophora	0	0	0	15	0
4bc7d8fcd306669cdc055dff9ee71467	NA	0	0	0	15	0
185aed0143ba36b8388ce8a00c769e14	Dinoflagellata	0	0	0	15	0
0ae61faa7ce4469079a4e0544c6945a2	Apicomplexa	0	0	0	0	15
b7fca8d67ccd97ef5f47de99c0ab266d	Fungi	0	0	0	0	15
7c80d09c050968467555523b4a258891	Ciliophora	0	0	0	0	15
4b9d83a9c69d8bc63e72d657a3515d75	NA	0	0	0	0	15
fe961b074960a9cf1b1fb20976afd3ea	Metazoa	0	0	0	0	15
ab9a325eccb31cc914aa9c362f73a4a8	NA	0	0	0	0	15
c54edc055504409827eb37adbf2f7195	Apicomplexa	0	0	0	0	15
61499654b10416ac892a746283a4c315	Dinoflagellata	0	0	0	0	0
d27dabfbdd4aafb3fbf9ce5debc1e757	Ciliophora	0	0	0	0	0
d9b72559b6bea22b27139106e2496270	Eukaryota_XX	0	0	0	0	0
ed1b8941dcb49fd1b7656437fd52e90e	Dinoflagellata	0	0	0	0	0
3d90741efe898022a4657adb8c0851b2	Stramenopiles_X	0	0	0	0	0
a95a436458d12284d6b539a0698b226b	Cryptophyta	0	0	0	0	0
f323078e8a4696c98719ed3f1c3588cb	NA	14	0	0	0	0
a490dd1049b8c2eee059f63ccef922b4	Ciliophora	14	0	0	0	0
1df3652daf57750607e47cb8e9057126	Dinoflagellata	14	0	0	0	0
669691348be350072f66da8d1c8627de	Ciliophora	0	14	0	0	0
bf50b7a8db7c4289cc1d27cb751bd900	NA	0	14	0	0	0
818e6d5e87c77465c50eae1c1c22fd0b	Dinoflagellata	0	14	0	0	0
ea96baece3c3c25e64ffe113799bf35a	Dinoflagellata	0	14	0	0	0
2927f8f627bd68ae1990205548812e84	NA	0	14	0	0	0
4a1faf9d90edec1ae90380db39517175	NA	0	14	0	0	0
9693bbda539771f76b8fbbb59ff123e3	Dinoflagellata	0	0	14	0	0
1434062b52fce37633267e170441191c	Metazoa	0	0	14	0	0
c7fedf26af6a477591bffe78a35b8f2a	Dinoflagellata	0	0	14	0	0
33cfb496555a6c686459cb6756e4349f	Metazoa	0	0	14	0	0
2c7a603b9f0edbb9dff6bd8b0d205133	Dinoflagellata	0	0	0	14	0
8b038bfe5efe891af4b42c79e0f878a5	Dinoflagellata	0	0	0	14	0
db4c6f13169aafba44d1fa5a3a5efec1	Chlorophyta	0	0	0	14	0
ba93c2bda5f68d14df9fce3157547e8b	Ochrophyta	0	0	0	14	0
e6c6eb29aed190a47f92f3dd5481f6b0	Dinoflagellata	0	0	0	14	0
c96f4803d6b00df6d953b6eb8bc3aefb	Dinoflagellata	0	0	0	14	0
21ab9a492a32443c64bcaab71e6ec5f8	Cercozoa	0	0	0	14	0
c927f98ee3de25c4794d92dbc80a0c98	Dinoflagellata	0	0	0	14	0
d73910fc18c6791e1a423a47a5469b10	Dinoflagellata	0	0	0	14	0
75cf226270eeeff3d9ac923c09daa1d2	Chlorophyta	0	0	0	14	0
1de82efef8d0ef88724b5bc839cfbb61	Dinoflagellata	0	0	0	14	0
874f8dcd799f8970de9ae909f6694f94	Dinoflagellata	0	0	0	14	0

1c78551111ac2bf1ed48284b2772879f	Ochrophyta	0	0	0	0	14
640324e7d374e755286515ccf6d586ca	Eukaryota_XX	0	0	0	0	14
9e157c51f9f2b161413bdbf2a02ab1c5	Eukaryota_XX	0	0	0	0	14
9e550bc7bf6b7935b9754e00bea3bfd0	Cercozoa	0	0	0	0	14
5e61321af45314e9af677e3e79d7a3f9	Dinoflagellata	0	0	0	0	14
3f45a0104cd87d94e955f7c3001ddc75	Ochrophyta	0	0	0	0	14
9360ea116d84533f896681cacff9fa25	Ochrophyta	0	0	0	0	14
d023f30f26ec5791dd150c0bb4a6c0ee	Dinoflagellata	0	0	0	0	14
1eb3abceb5857cef1f03246cf94aaaff	Dinoflagellata	0	0	0	0	0
b9a5ebd897ef3544d69de23d056fc144	Dinoflagellata	0	0	0	0	0
5a432d7b62b238f2ffff73a6d9779476	Dinoflagellata	0	0	0	0	0
80ffaab7b3e19e9e3be08b6bc1efaf2c	NA	13	0	0	0	0
ebcb5d73309e073c0d4a5225aee7d565	Dinoflagellata	13	0	0	0	0
2e115f6b769844d63a07be7d9ef829a3	Dinoflagellata	13	0	0	0	0
4dae1745fbf19335b58ef969011df3c3	Dinoflagellata	0	13	0	0	0
b1ac11591e66e2c529d356d0be8a3005	Dinoflagellata	0	13	0	0	0
df8a9e3b6664c2f0c5e2ca47e26c5643	Metazoa	0	13	0	0	0
04a9a9e3d7482d4a09b34a24193612f5	Dinoflagellata	0	13	0	0	0
96d7af33d6c7c05b9035be4021299aa9	Dinoflagellata	0	13	0	0	0
af4bd178f25810d891070bb8629c1706	Dinoflagellata	0	13	0	0	0
d7eb2580cfd841ca9f5e56127b7df76f	Dinoflagellata	0	13	0	0	0
3e83565784d06718edfa5215bb0d9d88	Dinoflagellata	0	13	0	0	0
254fec73ce0fbb993971864b3fd07217	Dinoflagellata	0	13	0	0	0
398c029bb249d144436b459ec8375f80	Dinoflagellata	0	0	13	0	0
390373b4c22d1ef9344ea388c4be99e2	Dinoflagellata	0	0	13	0	0
3cb258bdfcd9bdcb298291562696c9c6	Ochrophyta	0	0	13	0	0
9155331b37f1173cad727479e3107d99	Dinoflagellata	0	0	0	13	0
dd1d42d58daaa014c68043257585fe69	Dinoflagellata	0	0	0	13	0
502ed00f2665c187848809069e04af4d	Ciliophora	0	0	0	13	0
fa35c099c7dca5c443139ae9b5584173	Haptophyta	0	0	0	13	0
e55581f3d6b20f24fee031f066423720	Chlorophyta	0	0	0	13	0
a457760671be8bf4200812f5cd0061b0	Dinoflagellata	0	0	0	13	0
604be9bc053e8fa67c11a558e91f06f5	Dinoflagellata	0	0	0	13	0
bea81d37df871f5e720c88e8c1cb1314	Stramenopiles_X	0	0	0	13	0
763c412a558105c5f39b10c7ce343566	Dinoflagellata	0	0	0	13	0
e25823da6e53af79a94bad199174fccb	Dinoflagellata	0	0	0	13	0
2f309a4a9163f882dbb06051b4707079	Dinoflagellata	0	0	0	13	0
5ae53b80d059898da989c60512a8751d	Dinoflagellata	0	0	0	13	0
738091ae08159ac42321704503ea064a	Dinoflagellata	0	0	0	13	0
ec72c31e782c023620682dd5b013faf3	Ochrophyta	0	0	0	13	0
bff8f59545c34b8072cd92292607e6c7	Dinoflagellata	0	0	0	13	0
3a15f7445824e8d1b5738b9674b72986	Dinoflagellata	0	0	0	13	0
03789592d81ef1b07745bbd98457991f	Ciliophora	0	0	0	0	13
7afede44aba66dc32c30f1e8464df794	Eukaryota_XX	0	0	0	0	13
9ea2689740144c7773c03f6493ce65ed	NA	0	0	0	0	13
9f33145abe74cf9dab0be300be2fc2be	Ciliophora	0	0	0	0	13

1856a0a864ab76d1a81fc40ee28efa06	Fungi	0	0	0	0	13
56f0a3745bd6d9f02d4d627207057ae0	Ochrophyta	0	0	0	0	13
53fefc15afb492ca6ff0106cc4093953	Ciliophora	0	0	0	0	13
0c7e5d78997f5c7ca0cf433598557d2b	Metazoa	0	0	0	0	13
6ffd9e0a94735f109268d3ce247a14f1	Ochrophyta	0	0	0	0	13
8dac4f3beed3088a2f96ece2b340760d	Choanoflagellida	0	0	0	0	13
2eea44a1ee8f3cd31b5974b6f010aa1f	NA	0	0	0	0	13
298cea1e6a65f92ff955096e1e3fd063	NA	0	0	0	0	13
943c8dde534e3e2113ebb547f49a5e4f	Stramenopiles_X	0	0	0	0	13
fe61c6432b68f3a158c052e5ae61c936	NA	0	0	0	0	13
015b9199aadc6a3d99ffb6f058be3c1b	Ciliophora	0	0	0	0	13
809b159f44292020e5e424eadcf255f3	Ochrophyta	0	0	0	0	13
215a8bf8242034c38956a26e197a170b	Eukaryota_XX	0	0	0	0	13
7d387331035573dd5c9517578938d6cc	Dinoflagellata	0	0	0	0	0
f971f0ec1a857c43ed50fcb5e85c18a9	Ciliophora	0	0	0	0	0
6a95ba750aba107f719be510401c938f	Dinoflagellata	0	0	0	0	0
c227665fe24320a0772107991d9671ed	Dinoflagellata	0	0	0	0	0
be719c1629f9fadb0b8599be9da04b8b	Ciliophora	0	0	0	0	0
e49e1e3b48afc2a8058c3c238445ce63	Haptophyta	0	0	0	0	0
361838be8ebaae008727652d913d833f	Cercozoa	0	0	0	0	0
8b4478f78ba936532bc0caffe33ac395	Dinoflagellata	0	0	0	0	0
ccbe9debe2d4d51f5af49179a3f50b1d	NA	12	0	0	0	0
e0a86f52626f2eb74aa668a0e51452e3	NA	12	0	0	0	0
027020ed953f80f450c8c6698a6f38a2	Dinoflagellata	12	0	0	0	0
b8ea0ba8443358ded79368902f16ec41	Eukaryota_XX	12	0	0	0	0
6a6bf3bed684e4f4bad5daa9068525da	NA	12	0	0	0	0
b05c879f7226875363ad30a031f4f8bc	Dinoflagellata	0	12	0	0	0
616e4231a9ddb3c471cffe98db0c7fa9	Dinoflagellata	0	12	0	0	0
075288fde13a2bbedfa7955018c240b7	Eukaryota_XX	0	12	0	0	0
28228187845b9e5bcb21224a77d538db	Cercozoa	0	12	0	0	0
9a2a53014776c5521b15e3f200399d29	Haptophyta	0	12	0	0	0
4a57cacf08b3e302d7674f5e286e1d9f	Dinoflagellata	0	12	0	0	0
6a1347737b147c5d9ab1a2151fd4f2ed	Dinoflagellata	0	0	12	0	0
aa748af2d6a43ebb388e82a4a2e9e439	Dinoflagellata	0	0	12	0	0
214444230b443d89e5ae96663f3e6501	Metazoa	0	0	12	0	0
e30ac5d28af939909945d7e8628582e0	Dinoflagellata	0	0	0	12	0
fd471a8f297f78bdcaca22231ef2a0f6	Dinoflagellata	0	0	0	12	0
1b02d39bc43bd73f3ff316e24039e014	Dinoflagellata	0	0	0	12	0
6f9eb613c92d2619930fd6997e5f7eb7	Ciliophora	0	0	0	12	0
314225faa043f3ec3d86e120c733882b	Metazoa	0	0	0	12	0
eecc95c45f1ab5dfdb92f18995d2d074	NA	0	0	0	12	0
da1eca04610f9d8971d8c50a2bf8b448	Dinoflagellata	0	0	0	12	0
a425ef506885762698 6180eefeed4843	Telonemia	0	0	0	12	0
88dc2d4ee020a23756ce5e5b494a5a31	Dinoflagellata	0	0	0	12	0
51029892d20bd63e95869a52d3346272	Dinoflagellata	0	0	0	12	0
56ab83ea627503e9b7f74506d4de4b85	Katablepharidophyta	0	0	0	12	0

6073e134cc6205aadfe30c0d40305d55	Dinoflagellata	0	0	0	12	0
7229585c7de7bc62032c33e74c2816cc	Fungi	0	0	0	0	12
1385b065f22ef460ed3b3a0a6772f2f7	Dinoflagellata	0	0	0	0	12
aa4b7b2bd9064cb7a5002fd3575e1a5e	Ciliophora	0	0	0	0	12
b84da65f1cc69191d69f96a31d3c3ed1	Eukaryota_XX	0	0	0	0	12
0948075bcf57fb8bf823272bb871b853	Ciliophora	0	0	0	0	12
94a8f21e0e55c9d6205b2c6d24f39623	Ciliophora	0	0	0	0	12
cb823c22d211e12b6143775cef71a0c7	Fungi	0	0	0	0	12
20b58a53a41a659d47aa3122ed88694e	Stramenopiles_X	0	0	0	0	12
310b6078737e831dfaee85dcf4e10f24	NA	0	0	0	0	12
a4d9de5421e988dc9e8a0e1c316e016f	NA	0	0	0	0	12
0fd37c2aee7a67ea19546c09c866b4af	Dinoflagellata	0	0	0	0	0
0b97b37f7304f460348ff5dbf210da5c	NA	0	0	0	0	0
2aa6717aeb206a5fe9c22cf5102c07e7	Ochrophyta	0	0	0	0	0
91c426f6fdf6f890b7f3867dc515db0c	Ciliophora	0	0	0	0	0
5ec472df0dd6dbcdda8e96422407896c	Eukaryota_XX	0	0	0	0	0
865914454f0fb3a4fd360f08402f3c00	Telonemia	0	0	0	0	0
a92096c46339099d3048cdc11545c24c	Dinoflagellata	11	0	0	0	0
76ba85c408fbfc5f4984eaeec83e046e	NA	11	0	0	0	0
48b89b08dfed5668cf8d3b37528bbc1b	NA	11	0	0	0	0
f9b249df54ff9f478e2fb71c3862e4d7	NA	11	0	0	0	0
7578d1302bbbfa5b7928118962bba6cd	NA	11	0	0	0	0
97c35edd7459f7edfc933fa43435667d	NA	11	0	0	0	0
687a8ac7fdf1bc3ffab4adf377c73e76	Cercozoa	0	11	0	0	0
e79215a981009c7e6527230aff8e3fed	NA	0	11	0	0	0
15f03c0e2f8f3050e08b16cd6dffe414	Ciliophora	0	11	0	0	0
58bc1d684d6624b3403d9386b2dddb38	Ciliophora	0	11	0	0	0
f10733e960197a3ec2b9256cbd9f52fb	Ciliophora	0	11	0	0	0
ecb3c9734d7f9ede7ac7e371cfd55e80	Dinoflagellata	0	11	0	0	0
fdb9720f11bd526954c6546702def6c3	Ochrophyta	0	11	0	0	0
aa4e632578997c99500e9b91aba3fed8	Dinoflagellata	0	11	0	0	0
7f9ddc91fb2a3ce8fde378ad6b541efc	Dinoflagellata	0	11	0	0	0
952fc0e839cd85aee328168dc9164e80	Dinoflagellata	0	11	0	0	0
ae0ad1911073df1ca362052ae2bc4b5e	Dinoflagellata	0	11	0	0	0
811e3ad4c3c059444aa30e232a91b484	Dinoflagellata	0	11	0	0	0
2689cf74b09cbf9a5006e4673afe1226	Dinoflagellata	0	11	0	0	0
3b57e35c2ee45ca07873a4e0c67b3a60	Chlorophyta	0	11	0	0	0
11563d7210a477a71744415036543d68	Dinoflagellata	0	11	0	0	0
d6242249c53bd400ebe133ed83d1b438	NA	0	9	0	2	0
8e1704ee632b6e217b5702d52feff8a5	Dinoflagellata	0	0	11	0	0
11e6ebda8d8f89663ef79fb768ab760f	Dinoflagellata	0	0	11	0	0
d6371c53d083782c93259a6253d8a17e	Dinoflagellata	0	0	11	0	0
72329f0cb8f66ed72a26272b2b1b08cb	NA	0	0	11	0	0
eeb656efb919d1d50fc3aa107e198592	Ochrophyta	0	0	11	0	0
a0c2b7d95c787cd5756c8f6fb9312110	Ochrophyta	0	0	3	8	0
145050b7e290b33c32e980d2c82df4c0	Dinoflagellata	0	0	0	11	0

35f88c1ace0292dceac0fab6cdafa4ec	Dinoflagellata	0	0	0	11	0
ce9ca4fa7b07a2c097ff8d55b800e074	Dinoflagellata	0	0	0	11	0
9897713a3449852bab7bdcd3a75651fb	Katablepharidophyta	0	0	0	11	0
443a7e9bc343f6cf4c0ae3e58632f332	Eukaryota_XX	0	0	0	11	0
1cc4d4eda9eb44e79561fe4fd5bc84aa	Dinoflagellata	0	0	0	11	0
a345d4bac987ac97e29b9a22146ce0fc	Dinoflagellata	0	0	0	11	0
34ae250940f44244801757292d86d36a	Chlorophyta	0	0	0	11	0
af29f184eb1c31980ff3d7ea00cbbc8f	Metazoa	0	0	0	11	0
7bc7824915e4ad7f90510c1944465d25	Dinoflagellata	0	0	0	11	0
7a313f2512786a1dea028ffc6b713f31	Dinoflagellata	0	0	0	11	0
a89a959e462de137043bb800b21ccfbb	Ochrophyta	0	0	0	0	11
3fe77639e596a7e547d587da2c3605bf	NA	0	0	0	0	11
64132595851533fa11b4958eefa6b0f3	Stramenopiles_X	0	0	0	0	11
73555f616bb990aa6cd3313e4bd86e35	Ciliophora	0	0	0	0	11
8a57e83a2de932ead0fbb3054a294027	Dinoflagellata	0	0	0	0	11
0af64ba00f0d9fe8e68f1c7ee4972034	Eukaryota_XX	0	0	0	0	11
1f117dccfe6d450a1d3f55604af1d910	Fungi	0	0	0	0	11
45306fe0933482a797b4d43ecc9d97c7	NA	0	0	0	0	11
d39bb605991cb9083a224a7cdefd5833	Ciliophora	0	0	0	0	0
6f404d43e6887ad76cea7ef5c9410ba9	Dinoflagellata	0	0	0	0	0
787e6b1107f0fc82de2fbf05a052935d	Dinoflagellata	0	0	0	0	0
99e24f09bb9729fe154996c75cb83366	Ciliophora	0	0	0	0	0
f84397e72ad543de681b4b811789eda2	Dinoflagellata	0	0	0	0	0
2defa5c5de7fa45699ed552f7e125c1c	Metazoa	10	0	0	0	0
69fe8a8e75c3f76d20b087aac5ac2108	Eukaryota_XX	10	0	0	0	0
f52b98d7f4c19ba445c89b94fdd7c493	Metazoa	10	0	0	0	0
46081cfbba356f4aa0f638e5c5202e62	Ciliophora	10	0	0	0	0
4b99c915b8301e7bc22453240855add8	Dinoflagellata	0	10	0	0	0
6165b7a10f4eb50bdf77b2838aefe7eb	NA	0	10	0	0	0
ccf396c4566bc1b35ff49ea5f793f480	Eukaryota_XX	0	10	0	0	0
d71f65803eeeb0bd0a1af8b7d28e1c2b	Choanoflagellida	0	10	0	0	0
35fd63e7abfb7faabde8fb3c251348cf	NA	0	10	0	0	0
b7deaf566efc0964126f14778d791184	Dinoflagellata	0	10	0	0	0
9f03338a3fdda082e8f0f77a5888aa24	Dinoflagellata	0	10	0	0	0
36abba7aff0fc9ed8506ecad8b063886	Fungi	0	10	0	0	0
5700cea936a588ab4e55de73c0371702	Chlorophyta	0	10	0	0	0
e803895d00734acd5f97d3449c60be4a	NA	0	10	0	0	0
3196f92aa72928c1795634d54eb4865c	Dinoflagellata	0	10	0	0	0
08bf114b9ab9b63fe612d4850ef736b1	Stramenopiles_X	0	10	0	0	0
e8685494259b3e1fd2b5c6b179742c5a	Ciliophora	0	10	0	0	0
e76c472b8bb6faecbef981ee5520d2b3	Dinoflagellata	0	10	0	0	0
960ce867c77a11edff4d386a9681d358	NA	0	0	10	0	0
c27b80a620eda9c5c0ae5c9459510f6c	Dinoflagellata	0	0	10	0	0
f5f9e4a1873095154bd17a47c6541601	Dinoflagellata	0	0	10	0	0
97e651d7bcee1f635b5bceeee87e28fa	Dinoflagellata	0	0	10	0	0
383e581cf61a6751f15272d426a700b8	Apusomonadidae	0	0	10	0	0

fa1cc4debe83236e18483e16a19c5e5e	Eukaryota_XX	0	0	10	0	0
a9400ec5d07c8e9a6f52a9bd750b1605	Dinoflagellata	0	0	10	0	0
24230b47fdb7f15db900e53ee76fd7a4	Fungi	0	0	10	0	0
7c20e5cbce805873da7dba2427d6766e	Stramenopiles_X	0	0	10	0	0
d7b91c060da20d0e86f1df29edb65bca	NA	0	0	10	0	0
7dfebd5b2b40ecc31eef6fd15a5972a5	Lobosa	0	0	10	0	0
ebe26148d26270c0e22dc2801d61d457	Dinoflagellata	0	0	0	10	0
93d49be29f1d783103a4894e34b62b80	Metazoa	0	0	0	10	0
5950e97b38c65ea4945fb291512d83fc	Dinoflagellata	0	0	0	10	0
1c75408e1423b2771410015883c3ec16	Dinoflagellata	0	0	0	10	0
3134bfa87127cb1d06ab92757f8055c3	NA	0	0	0	10	0
bb03c2620b5d1410ef347d509d7ad023	Dinoflagellata	0	0	0	10	0
5396446e4b5f0f20703f0dc0136b79f5	Chlorophyta	0	0	0	10	0
adc6311b9ee3279af49d9469f1ab20a9	Ciliophora	0	0	0	10	0
740b3a9e221c3b8c4078ed1b54aeca3a	Dinoflagellata	0	0	0	10	0
7b61d3494d82667f3dd76834e154b4c6	Dinoflagellata	0	0	0	10	0
0b580a56e92f3d2ab7872cb161ec3185	Haptophyta	0	0	0	10	0
aefff967d7dddd89ca41b0c2e1afca56	Dinoflagellata	0	0	0	10	0
89f15fc01e50b2bbfec910779452c2a2	Chlorophyta	0	0	0	10	0
d2ea7780a0b3e055b9c4eca2d77ead11	Dinoflagellata	0	0	0	10	0
99865cb1dd0c69725dcd8505a0daa503	Metazoa	0	0	0	10	0
902ada321ed2373316d8b100d844b5f3	NA	0	0	0	0	10
9bf0f6422063ce65dfb9b635b693003a	NA	0	0	0	0	10
78c77d927055498d8878032ce7143636	Eukaryota_XX	0	0	0	0	10
a126a7ed9e7501a537ebb9d44cb8effb	Ochrophyta	0	0	0	0	10
a67c1b7211e8fe3e6fb867eb20fd2456	Dinoflagellata	0	0	0	0	10
4b6bbc6c64e354506d573a4ea9b085e1	Cercozoa	0	0	0	0	10
c7eee8cdddbd47a3b5aa78558cb405e1	Apusomonadidae	0	0	0	0	10
00ea09a2c7c1af0a4a009a3980f4027e	Ciliophora	0	0	0	0	10
52a7e64e1423f3d6d448a6ac4c2df616	Fungi	0	0	0	0	10
592ae84ae70b069a13ca8c12ac5c9623	NA	0	0	0	0	10
3ec6139e3e8a13ac9728c36f5c5b9a4c	NA	0	0	0	0	10
1075b0f6cc2915807ec58cc9a75b64f4	Dinoflagellata	0	0	0	0	10
bc35972ac774352075a6f9a8a8be01f5	Fungi	0	0	0	0	10
f6fdb6678cad413a6696d3ff39a06db6	Ciliophora	0	0	0	0	10
9443f8415142787e503c61d652388ee8	NA	0	0	0	0	10
0a873ae9e21e377dba7b54aebee20677	NA	0	0	0	0	10
31cbef73d43bf85912a0f20623caecce	NA	0	0	0	0	10
a29ab65ebd1a0ecdb15da1f6c6c6c9df	Dinoflagellata	0	0	0	0	0
55fcea898d5f48614ab59915bd463cfa	Choanoflagellida	0	0	0	0	0
a1df0aad2511679b95bda25312c366fd	Dinoflagellata	0	0	0	0	0
42b3449985736f79bfeadcadbc8b97e5	Dinoflagellata	0	0	0	0	0
6bd90122181eafb80de19918b6b84d68	Ochrophyta	0	0	0	0	0
7f8224df59da18d1f2a89c3394199f56	Ciliophora	0	0	0	0	0
a6c7d3c4de58b09fab55f9ffc7198e52	NA	0	0	0	0	0
ed5256d21afd7c1bd038f7faaedbcae9	Chlorophyta	0	0	0	0	0

a9ef0800bcd942db64732c0243d4abfc	Dinoflagellata	0	0	0	0	0
94b31e88c6632607f9fd548389361122	Apicomplexa	0	0	0	0	0
04d2d883c095466d051237cf6c948afd	Apusomonadidae	9	0	0	0	0
9571ed23104ff28c07d6113f7f8ef79d	Eukaryota_XX	9	0	0	0	0
04cf5bfb05ad20e6a5f326c7772dddb9	Metazoa	9	0	0	0	0
29fd7b3f8b4a1d690ada74e892e34fcc	Fungi	9	0	0	0	0
cdf85c7fe1bbc57a8c9bb68e66912798	Metazoa	9	0	0	0	0
b7658f8193ed03efe46efeb2907948c8	NA	9	0	0	0	0
7de4db571acac24872c46b4cb48d1ac6	Dinoflagellata	0	9	0	0	0
0791e07f4edc13ea9fe296ddbb11c600	Ciliophora	0	9	0	0	0
a84e0c92f20cf640cab90a6848a4c86c	Dinoflagellata	0	9	0	0	0
89bb9585aa3805a428c70e1faf348e46	Katablepharidophyta	0	9	0	0	0
ae66de5bdf9f36a4fa53b32c860d45b9	Eukaryota_XX	0	9	0	0	0
ec976a2cf9cf20b602634fbd4b7c28c1	Dinoflagellata	0	9	0	0	0
3a464fec0643bb007a6a3edc5ad32a9c	Dinoflagellata	0	9	0	0	0
7b924d34afca35c34c979ce6e614071c	Ochrophyta	0	9	0	0	0
d40f83fa2b3c10d106fc4f4a4c4b6850	Dinoflagellata	0	9	0	0	0
0a0783a895abc0a84b46ff0d9ff68079	Dinoflagellata	0	9	0	0	0
fdd8fd82578b3a125f4196555bec5f9c	NA	0	0	9	0	0
8b65845ccab80f4af8ac5d5e2e63f155	Metazoa	0	0	9	0	0
ad82fbe21ff7cb6987ac994d63dde5c0	Apusomonadidae	0	0	9	0	0
a175fc5fbe46659fad92ee160eec2e6b	Dinoflagellata	0	0	9	0	0
4d7843517fce9c1405bdfda08750f1de	Fungi	0	0	9	0	0
922bef58661a1678259d626647e0754b	Dinoflagellata	0	0	9	0	0
a5092b1efc74a2712575dd7cdbd11698	NA	0	0	9	0	0
63e0dcaf6f51dcb6b6b3985194ed681a	Dinoflagellata	0	0	9	0	0
f7bc3c9d007c90fcfb1898170765bbf3	Dinoflagellata	0	0	9	0	0
fffa4e72d4b7bf43ca7381d8ce16797c	Dinoflagellata	0	0	9	0	0
a0635257e6db86db61817a58f8ac220c	Dinoflagellata	0	0	0	9	0
1132e3a8c8bf32e1cc66fafc7b79afc7	Dinoflagellata	0	0	0	9	0
3362419da69c51ee7e5e1f341f0787cc	Ciliophora	0	0	0	9	0
ae7bdb8cb6b7e55319515c588bce5be6	Chlorophyta	0	0	0	9	0
947ed246e792eac1235747bc340b29d8	Dinoflagellata	0	0	0	9	0
aff91b40e85d8b0dafad1d357deb4910	NA	0	0	0	9	0
adbf08f8d4493d049250b1e04464d1d8	Dinoflagellata	0	0	0	9	0
b62d0d8f65c619dd49be91bc20e5bd55	Stramenopiles_X	0	0	0	9	0
a50f5295edf0b6802322f7aebd6a1d8a	Eukaryota_XX	0	0	0	9	0
7a7cc9f449cda9563cba0af65a2e673b	Chlorophyta	0	0	0	9	0
778051064fda61637abbecbf434eced0	Chlorophyta	0	0	0	9	0
c2cb95988d230f34239f83fb645bb161	Katablepharidophyta	0	0	0	9	0
fb9fd41942ea7d6fbe293267d640eb28	Dinoflagellata	0	0	0	9	0
b6641bbbcf181f349db3b732cebb5c94	Fungi	0	0	0	9	0
739b46bdf581b6cb87597885f12e40c4	Dinoflagellata	0	0	0	9	0
1c2d591807aa893de5f0c7760ce464d5	Dinoflagellata	0	0	0	9	0
79d064cd6b29f86ebb725d221bf48185	Eukaryota_XX	0	0	0	0	9
595ce0ea719576892b80870d956d6ec2	Eukaryota_XX	0	0	0	0	9

8e208667bc88a3e9b9b36f975cd29eb8	NA	0	0	0	0	9
e1142a4c847c02bb7a83678bd3675a08	Dinoflagellata	0	0	0	0	9
fa7c1899b546db1215c04da7531ae79d	Stramenopiles_X	0	0	0	0	9
a0b7391c4737a2b4a931f5b92a3ff209	NA	0	0	0	0	9
5a64d74ac8481c1d3cc9ee496c5fd6ae	NA	0	0	0	0	9
b48ec29ea4a4936e5d627a385cc5c92a	Eukaryota_XX	0	0	0	0	9
7fe87f0496c778c31c6b11ed9b99bb04	Eukaryota_XX	0	0	0	0	9
143b1ea3099d532f1b013568213d40d6	NA	0	0	0	0	9
951c5cd512b9606997bb61ce3936544f	Ochrophyta	0	0	0	0	9
c334aefde2c22963f80cd0dff07b89c6	Eukaryota_XX	0	0	0	0	9
313c94d75b3fdd0c5d2f3ec1aa2fd35b	NA	0	0	0	0	9
cb097915200afaa1a90809fa5cce88ce	Dinoflagellata	0	0	0	0	9
3222d30a4c45372084a01b322be03a63	Mesomycetozoa	0	0	0	0	9
428d069842a5078ffb76b6e6961882b0	Metazoa	0	0	0	0	9
50f2f6e4c3fdc632ef3277e92ae07946	NA	0	0	0	0	9
cb37deb9e42935ceafbb2f5693a64b12	Stramenopiles_X	0	0	0	0	9
24217dbb9f3063b987ff668b1654ac67	Eukaryota_XX	0	0	0	0	0
e16fb605b75c509c6818d3257c636401	Stramenopiles_X	0	0	0	0	0
90fc7a7bdb7f0b221f3b94b5ec99cf7c	NA	0	0	0	0	0
2834df22923900504135dd5eae9a3877	Metazoa	0	0	0	0	0
9f2a2f3ac7811f61f80de47d3d38bea2	Katablepharidophyta	0	0	0	0	0
5f6fc8d848676c4f3eb5e1b9a7d17483	Dinoflagellata	0	0	0	0	0
0fc0a9da4fd4ca3b471f97a3c49bde73	Ciliophora	0	0	0	0	0
f9a8b312f3ff1035f729898135f1d44f	Ochrophyta	0	0	0	0	0
69c2e7ea823bd7a4fbdd84fd0a29cce7	Eukaryota_XX	0	0	0	0	0
cfcb7f238fdf0664d4b50eae0da4da86	NA	0	0	0	0	0
54c82807e008f7a66d9952e62d9c00a0	NA	0	0	0	0	0
197b13f23bc9bb45f0fb08abd08b35ac	NA	8	0	0	0	0
6eb2f187690b66a1772adabd36096dc5	Ochrophyta	8	0	0	0	0
c5b98f3ae3733bddec07bf50929eec12	Ochrophyta	8	0	0	0	0
15dd5194912202461f8128443cd4ecc7	Metazoa	8	0	0	0	0
905b92f274ef621b09a0fe32d2861271	Ochrophyta	8	0	0	0	0
a87ceee637f9a069e7421649abfa35a1	Ochrophyta	8	0	0	0	0
64691828340873c61e50c8d8990d0076	NA	8	0	0	0	0
e0e0340968cecf27292b17e20fb8e38d	Metazoa	8	0	0	0	0
8b2e331f52b6c76b7a4a46eac7b41c62	Metazoa	8	0	0	0	0
faffe8df8bf2b689c8ddb9f5856eff14	Dinoflagellata	8	0	0	0	0
ebe8aa27a7fc4991da64229af207a704	Eukaryota_XX	2	0	6	0	0
726fcec82f954f07e950dcf5e705f193	Dinoflagellata	0	8	0	0	0
e8a955a1c57259e16154a26772f1883e	Telonemia	0	8	0	0	0
9faf99ea927bc56f9935b628ec1722ec	Dinoflagellata	0	8	0	0	0
7e4e4343047b294c82ee75a99c95297f	Fungi	0	8	0	0	0
1d647cc76a5b42bb15677862c0258fba	Chlorophyta	0	8	0	0	0
f7e085cc33538c22ddc1c6d1eb5b99e3	Dinoflagellata	0	8	0	0	0
98476ff1f1bff3bd852784f628d09451	NA	0	8	0	0	0
1c36ef392d5378136a7828d58cc94a3e	Ochrophyta	0	8	0	0	0

58c07c3cff713e0abce928e9e32bd5b7	Apicomplexa	0	8	0	0	0
19a73b157453df2851b3dcb027895276	Dinoflagellata	0	8	0	0	0
e976a937f8abff24ffda88d58eafbe48	Radiolaria	0	8	0	0	0
83f4eecf0cfd95110fca82295f9eb6c3	Dinoflagellata	0	8	0	0	0
987a63359b0cfc18c2730e8389faae32	Chlorophyta	0	8	0	0	0
4dd746bb177af4a885243addd949515a	Ciliophora	0	8	0	0	0
5344abbad43c73b269d546ffa0bb25c6	Fungi	0	8	0	0	0
c9a6e378c9fe8da07178af2b72586ee0	Fungi	0	8	0	0	0
dd84db38673c0216e3401d17174bad54	Choanoflagellida	0	5	0	0	0
5d5f1e15bebd43cff0e5a6c773053d70	Dinoflagellata	0	0	8	0	0
ca114554ad6c05098443441f044eea1d	NA	0	0	8	0	0
ccd1ee8541f1e56b2027bed4b4bbf219	Dinoflagellata	0	0	8	0	0
38fe311399770ba29621c762bc7ea154	Dinoflagellata	0	0	8	0	0
01dd4b89f47cc66bfef1ca6094a9ddde	Dinoflagellata	0	0	8	0	0
bf6fcfbb663520fca8c3f1858b114283	Lobosa	0	0	8	0	0
34906ce8abde5c51407c2056332c5609	Dinoflagellata	0	0	8	0	0
a6655932e191420ed7425a06faf35d66	Metazoa	0	0	8	0	0
23829922dfca61cafc10cb0fea70cb1c	Ochrophyta	0	0	8	0	0
5e12c8bd4f36beedc3fa504060210bbb	Dinoflagellata	0	0	8	0	0
217244d4542b402ea2af4ff943f351f2	NA	0	0	4	0	4
43e260619c512b58306e61c0716a2387	Dinoflagellata	0	0	0	8	0
7890f72adbb712f0313bdd52a8dff9e1	Fungi	0	0	0	8	0
9bf00f109c70a8afefcd6bca0f5570ae	Cryptophyta	0	0	0	8	0
968429cbb46ec363a3b6f52274aee6be	Chlorophyta	0	0	0	8	0
bc56c76793b56213a29b134458d43e0b	Ciliophora	0	0	0	8	0
31f6d38de3dc81c779bfc25de2b35aae	Lobosa	0	0	0	8	0
f218a1131245dec0f1034611ae75d930	Cryptophyta	0	0	0	8	0
29aff8f92bbfcb858194ebfce7cf3177	Choanoflagellida	0	0	0	8	0
7b7463e7f0f79cdf82f0c947154ca59f	Katablepharidophyta	0	0	0	8	0
6a7a589fee96682bd032939fe936fa6e	Haptophyta	0	0	0	8	0
117d0514eb09e27c202a137c5bfa63cd	Dinoflagellata	0	0	0	8	0
c3a9f680ddff2afb6777db9de06b956a	NA	0	0	0	8	0
66e8f3d9a82630375c6f4ace8df2e316	Ciliophora	0	0	0	8	0
669d632b5dc16663002ac2e670e94bf2	Dinoflagellata	0	0	0	8	0
d0a74312832ba82bd911b2635b92e1a0	Dinoflagellata	0	0	0	8	0
8be921f62a11703c98b16598501269e4	Cryptophyta	0	0	0	8	0
2a0c364c655348e6f51ccc4c273081d2	Ciliophora	0	0	0	8	0
524591fe534bf26bfb48831e3e354ed8	Dinoflagellata	0	0	0	8	0
23eeddf5ef7480bcc22f6108ddfe6ada	Chlorophyta	0	0	0	8	0
6182138dc09e2cc6f156b3d1e531895b	Dinoflagellata	0	0	0	8	0
f86d3a654b342ab056ff1bcf70141362	Dinoflagellata	0	0	0	8	0
dfdf00b6f89625c3da12c99afaad74c1	Eukaryota_XX	0	0	0	0	8
a89db8405d870f70de64786c912db95c	Ochrophyta	0	0	0	0	8
688811893c3baad248fcbca1f3b187a7	Eukaryota_XX	0	0	0	0	8
1f6d399d688df4766ab3caafdabec82c	Ochrophyta	0	0	0	0	8
302d0d4fc7e36e43ba8f346ecc220217	Eukaryota_XX	0	0	0	0	8

cac1d49c870c8965234bb817d4d2fa9c	NA	0	0	0	0	8
d258f1f7ba9b2492907351c1c9bdbb84	Ciliophora	0	0	0	0	8
340d87020393d1c69275fe927bdcb4a6	Apicomplexa	0	0	0	0	8
fa5f51adb56f86424c0cdabce7c5fe24	Eukaryota_XX	0	0	0	0	8
07d59f45acad05f318de248df1998c1e	Lobosa	0	0	0	0	8
4231ff404b76c4d4e0d4ac523158cf9f	NA	0	0	0	0	8
ff5f8e42f1bba4c69af590a63c79781e	NA	0	0	0	0	8
3c913efa9d45105197037551a07abf10	NA	0	0	0	0	8
742b55b24912458cc4fad0dd14c479cd	Stramenopiles_X	0	0	0	0	8
ca98a15d18be9a3dc9c0954fa301490d	NA	0	0	0	0	8
72f8806b24507d48d1a24be432352456	Eukaryota_XX	0	0	0	0	8
568ff6bb70edfccd623c393768ab610c	Dinoflagellata	0	0	0	0	8
6320356baff23c61aa71ee1fce927416	Metazoa	0	0	0	0	8
3ce30f7929695dff1cae4a387b22774e	Ochrophyta	0	0	0	0	8
b92dcbc141a5bd9ef3a4dd5f76566537	Choanoflagellida	0	0	0	0	8
a8c690aa4c7b0c1a549894bd3f5e56a6	Eukaryota_XX	0	0	0	0	8
b7e3db2d429ef1d87a78673248609f5b	Apusomonadidae	0	0	0	0	8
7438044a33fa61d74dc295c0223c8684	NA	0	0	0	0	0
ac5b148fbb75e3c665c81c244c5e4ebb	Dinoflagellata	0	0	0	0	0
e25998910749c9194657f83274413d43	NA	0	0	0	0	0
27d10368bc1d33985675051f5065cf2b	Dinoflagellata	0	0	0	0	0
6c1c84f704b5270eef73b569f2be0c6c	Cryptophyta	0	0	0	0	0
56f332251d82ddfd3fdcdcf899a626aa	NA	0	0	0	0	0
2a3f3263bb26a024d3b8bf36834a7bf8	Metazoa	0	0	0	0	0
58e04926ead84233ae513425af7be87d	Ciliophora	0	0	0	0	0
57ff9f42fcdab242836d643aba02344d	Ciliophora	0	0	0	0	0
d87e1ee73c126fab32ec41057d999706	Ochrophyta	0	0	0	0	0
a500173270537d90cc1ff4a5bb09bdbd	Choanoflagellida	0	0	0	0	0
77c64e3bf673693e11256f4ffc077ee1	Dinoflagellata	0	0	0	0	0
7edd0b23966681b2c1e89615fd6d0010	Haptophyta	0	0	0	0	0
5ee9d0db1a819f2b52b1d3423f8c5a02	Cryptophyta	0	0	0	0	0
d2d6f91bea08be0f6f5c0422d30a7332	Metazoa	0	0	0	0	0
c5d7cb7b7d0fc5207306d16b5c2684a4	Dinoflagellata	0	0	0	0	0
b10881504fb873f635c8c34dd79054db	Dinoflagellata	0	0	0	0	0
5b5e89787a7950684c518774b28d51ab	NA	7	0	0	0	0
5d9869987a8a9c45870555e8a0988abf	NA	7	0	0	0	0
e83f6a1266630f02cf7d6887779fb57f	Ciliophora	7	0	0	0	0
754c43bd4eb77c0fb0c3bca5c433615d	Metazoa	7	0	0	0	0
eed6c42583e896fd9e48fbd90c8ec8d0	Ochrophyta	7	0	0	0	0
796c6b9a92705213aae3042f659d496e	NA	7	0	0	0	0
279cec3d38fd5fa13e2c3a653d442c13	Choanoflagellida	7	0	0	0	0
0b685bd3a60a6ea212daf59bad912dc3	Dinoflagellata	7	0	0	0	0
c4b4452f42c5afc23b829bdc91cd1310	Haptophyta	7	0	0	0	0
e3fe627db2007eccc1b6427c2793792e	Hilomonadea	7	0	0	0	0
7b79063448eff6e72a6bb19edc01b36d	Eukaryota_XX	7	0	0	0	0
b6a57150d72766ae7db9a218c829fd21	Ciliophora	7	0	0	0	0

7b109385e86f2dff3981e862c26ca9e3	Apusomonadidae	7	0	0	0	0
e6e23e7e5e842fc906723e66a207ecc8	Ochrophyta	7	0	0	0	0
f685fdd8954bf4b82d6a1972ba5b9d4c	Dinoflagellata	7	0	0	0	0
a141905a27544860894bbb4a86c743c0	NA	7	0	0	0	0
63eb0718a1902664be8e65655805ff7b	Dinoflagellata	7	0	0	0	0
83f493e91aa78a873d59290362ac09f9	Ochrophyta	7	0	0	0	0
03d120c0204c96bcbfd6193bf22bd1e2	Eukaryota_XX	7	0	0	0	0
e8c13760481fb8542a9e5e61c1fb761f	Eukaryota_XX	7	0	0	0	0
cf7fae837b708319e5f473397b221a4e	NA	2	0	0	0	5
a80a1d53aeaf310a1a1f2939840285f7	Apicomplexa	0	7	0	0	0
a22b3e5a6bd8843a42e16331e6dfda59	Dinoflagellata	0	7	0	0	0
9b3e547a73d44199a2399efcdbbc33e2	Dinoflagellata	0	7	0	0	0
a5aedb23064bd77a54d9024c5042244e	Ciliophora	0	7	0	0	0
8e694643f133b8eb4275225291aadaa1	Chlorophyta	0	7	0	0	0
1ba038e0e332e63429b5779548487d49	Fungi	0	7	0	0	0
8805f3632fa8c9d4aa154cb836ed40c8	Dinoflagellata	0	7	0	0	0
5661023501ecb1350c30de9cc90b8a48	Dinoflagellata	0	7	0	0	0
e5bb810ff2d8bc0d89e6e6b6c8647416	Mesomycetozoa	0	7	0	0	0
71db88b80303452d277a8412d3a9b324	Chlorophyta	0	7	0	0	0
d8be330f1fa16ac16b4815c4fec47f8d	Dinoflagellata	0	7	0	0	0
0cc26c8e668223dbcf62ced6883a3740	NA	0	7	0	0	0
0274190f34603fc32f033d7823408831	Eukaryota_XX	0	7	0	0	0
10e58250ccf172f6ac0cb23e8c216e25	NA	0	7	0	0	0
acd573d35a84260772d8f862ed1e7721	Dinoflagellata	0	7	0	0	0
32cfc10cdbde27126e38c9db56d3255e	Dinoflagellata	0	7	0	0	0
6fab5c1b574c97468dd28bdba1d5ee61	Stramenopiles_X	0	7	0	0	0
1876e4badc87557bbe366b0bf30f7253	Telonemia	0	7	0	0	0
bde5cff17e3b49509698f5350ecf9cdb	Dinoflagellata	0	0	7	0	0
aeb46b38857df0ceedfc585205923ec6	Dinoflagellata	0	0	7	0	0
ca7e94a7f41bf98cc38b84c421a3af5b	Haptophyta	0	0	7	0	0
2055a776089beda09511132f570d5709	Dinoflagellata	0	0	7	0	0
4e5c8b9d1d34648c44b05b1c08777d1a	Fungi	0	0	7	0	0
b607eecc6c66795b5c83f248315bec08	Metazoa	0	0	7	0	0
aa5def9e9128961f23a66c5c612909e6	Dinoflagellata	0	0	7	0	0
f3aeff2c07ddb785a9c155a963e81e74	Dinoflagellata	0	0	7	0	0
72ac69da0bc3e3fcfcf381e6032b7e91	Dinoflagellata	0	0	7	0	0
9338bc42ca3804015f8638f051b3f86c	Metazoa	0	0	7	0	0
bac38834da5057f9b17f556a5628111e	Ochrophyta	0	0	0	7	0
44a61aa018cfbb9c35a38ad9c95404c5	Metazoa	0	0	0	7	0
d0fdf28ca1cf62c3033d150ed379657f	Eukaryota_XX	0	0	0	7	0
6ecd6a45c97ede26c537e878be76b4a2	Dinoflagellata	0	0	0	7	0
c1c7ffb260178f4a03bedf0ade60f844	Metazoa	0	0	0	7	0
0e1cf05bff2c58731c0a7a6ba2fca1ef	Ochrophyta	0	0	0	7	0
00e9f8bee95e090f484cd66942680ff0	NA	0	0	0	7	0
3d11cdede170d568c87db9b291285371	Metazoa	0	0	0	7	0
dbe80ff045215f84cbadab02cc870914	Ochrophyta	0	0	0	7	0

d810d99767f12a8052be6e26d2a8020c	Ciliophora	0	0	0	7	0
320457ed9d7aa526da1c5484c0c71fce	Metazoa	0	0	0	7	0
ce15d981384616262effcd932d3829b6	NA	0	0	0	7	0
097f4d830e420ea3731872c72d9b5593	Metazoa	0	0	0	7	0
3ab925ab9aefb7bcbc107a73550134bd	Chlorophyta	0	0	0	7	0
ccc45a4e4600d595658a967456370b9a	Ochrophyta	0	0	0	7	0
0a7b461746fbbbae0bab720b5eaf6fa8	Dinoflagellata	0	0	0	7	0
5737cadd4a72ee1da5eb05ce654b88e9	Dinoflagellata	0	0	0	7	0
6bf07bf014f6946a9204a08a89ec58fc	Dinoflagellata	0	0	0	7	0
bab4b9711deded3644412c12a7cbbc86	Ochrophyta	0	0	0	7	0
abb59136d020e6737c538948cc5f24fb	Stramenopiles_X	0	0	0	7	0
c3231cfa0001063182aa8f9ff2ea40a7	Chlorophyta	0	0	0	7	0
bde11b43096cc9db9e368e85b024ff6c	Cryptophyta	0	0	0	7	0
b040e59620fc1885c90296672b45541c	NA	0	0	0	0	7
f927949cf77c092d428d51db700487b6	NA	0	0	0	0	7
e8abac14b21e296c3df391d08439ec9c	Ochrophyta	0	0	0	0	7
bff741750245d494e49361a8f443fc12	Fungi	0	0	0	0	7
530cf0e1767d29920b75eb7277c0c820	NA	0	0	0	0	7
3b8cfdd9371dee629bc7b6bd1eb17191	Ochrophyta	0	0	0	0	7
2dd1f2053208075672db616ef0207d87	NA	0	0	0	0	7
69cbc3014a16f555359b6e4cfbffe85d	Dinoflagellata	0	0	0	0	7
4a4c5d461139751ddd86f3ce9ca9c586	NA	0	0	0	0	7
8220122c77e7824be18b9462a34ef022	Metazoa	0	0	0	0	7
bf86da5ffc94149e166bdd022d3c751d	Stramenopiles_X	0	0	0	0	7
e5168fb20c970402d13be9a232c8faaa	Ochrophyta	0	0	0	0	7
abf9b009e90b1327b617120715823294	NA	0	0	0	0	7
89b5ed7b9ddad151ef80da4a5982a6d2	Eukaryota_XX	0	0	0	0	7
b2a05e4429d754373fec95c952af33a4	Ochrophyta	0	0	0	0	7
37b547ee0dd0ee1684ec198a62e1fdc2	Telonemia	0	0	0	0	7
214a37ef710b2ca58fb7f0ba9f8e47bf	Apicomplexa	0	0	0	0	7
0bd8440e239f9503fecbeb1d556e5cf4	Ochrophyta	0	0	0	0	7
2bf85e2ea975365741eb7440b9a69192	Fungi	0	0	0	0	7
c33409a4c5a9e203a888fdcf6ec81c98	Dinoflagellata	0	0	0	0	7
ad5fa52411c6913c82b277c11649c309	Cercozoa	0	0	0	0	7
99ea80c100b2139727e14504d07155ee	Ochrophyta	0	0	0	0	7
606cbb17ade5b3c09dd301c30a628ea8	NA	0	0	0	0	7
2d2ea91948220f2387f593426d326058	Apusomonadidae	0	0	0	0	7
b1c3e95e8fe473cf124292c8f94d2bbe	Choanoflagellida	0	0	0	0	7
2fec0750d751015a637b5cf06e3a4cbd	Dinoflagellata	0	0	0	0	7
77cad5d1c1b7b93f0d1f5d77115370d0	Dinoflagellata	0	0	0	0	7
742b3eed3b710cf4d146bcb17bd1fe14	Apicomplexa	0	0	0	0	7
8a320360141617e4b0760f76acb4edda	Ochrophyta	0	0	0	0	7
781dadf1d418173941bcd7a65497bd1d	Ochrophyta	0	0	0	0	7
9d75690750c1787b9cdb9988f69ded50	Chlorophyta	0	0	0	0	0
1ba2360d1339a11b17fa289351fd84e1	Dinoflagellata	0	0	0	0	0
6bba88551773a5fe89f3b84e53b5c627	Ochrophyta	0	0	0	0	0

adeca58a466bc11634c956f431cd9c08	Dinoflagellata	0	0	0	0	0
f3167efbbf01d29e40c59a860cefbe75	Dinoflagellata	0	0	0	0	0
4b5050a91d09ddc17ba4c8bde7f95f10	NA	0	0	0	0	0
1b3b3755cc1cb58cc861c3a4e526a860	Dinoflagellata	0	0	0	0	0
6c157cf97b73ca6b200913bab420540a	NA	0	0	0	0	0
9252f7a538c7255cd276a567711d149a	Chlorophyta	0	0	0	0	0
72ee63fc702c25cf37fc9ad5f100bfa2	Chlorophyta	0	0	0	0	0
ea502cda04937a538cea4e69feab81a2	Eukaryota_XX	0	0	0	0	0
fd3fbc5da5f9a0f9e5fb3558508dff5b	Haptophyta	0	0	0	0	0
0bb686181e7fbd3f230cc4e285cf42d9	Dinoflagellata	0	0	0	0	0
4c60da2e5b6945efc4e2336c9f8f0020	Dinoflagellata	0	0	0	0	0
8c4985e9e7291f2db49e9fffd3f5f6ee	Metazoa	6	0	0	0	0
78084cb1cfd46d5c5f994cd7f730193b	Streptophyta	6	0	0	0	0
c2aff0768d67bf836b37e885f90a866e	Metazoa	6	0	0	0	0
0c57987887ab599b8fada9b208fc9893	NA	6	0	0	0	0
3f80007e50e6956201a59484c4bfa329	Eukaryota_XX	6	0	0	0	0
428c1c1f26c7d72cddc34dfb720a9482	Chlorophyta	6	0	0	0	0
3c7a9e45318610d30e1608a0b792b06a	Dinoflagellata	6	0	0	0	0
01236e19685c0e99bb7d762b8ed61d0e	Fungi	6	0	0	0	0
d18deb23c3848bf2402ac84e40a9fb61	Ciliophora	6	0	0	0	0
90c8d817e6c614a73008cecbc0bafa19	Dinoflagellata	6	0	0	0	0
5db975a5419b9db6e6544ba75bfa9738	Stramenopiles_X	6	0	0	0	0
a3ec39582209bfea93068357dabade6f	Metazoa	6	0	0	0	0
77921334f904ea2489e73824c2eb1226	Stramenopiles_X	6	0	0	0	0
f87d2774ccda58e1cf1b78263aac4011	Mesomycetozoa	6	0	0	0	0
990b2f065d14778186bc79888c4b08bc	Eukaryota_XX	6	0	0	0	0
e61cee2ee23c16edd1231c0a5344fe26	Katablepharidophyta	0	6	0	0	0
f4f1788f4ae7fc3bef11966dd1032204	Dinoflagellata	0	6	0	0	0
6a20c1ceeeeb2c2c988e680ee787a529	Ochrophyta	0	6	0	0	0
a5cfae08235a92d1f21492fff51828fd	NA	0	6	0	0	0
e0bf08c5662c12d9eff27a11ab07507e	Telonemia	0	6	0	0	0
9baafe0b9aabf1dba1e08784f52cea87	Fungi	0	6	0	0	0
bd47a7af417bebe854a800fe1a16f7c6	Ochrophyta	0	6	0	0	0
d7ac49366977cf8255736e2a849d86de	Eukaryota_XX	0	6	0	0	0
b65cd2f1679a97675130a7674b6d9b1d	Ciliophora	0	6	0	0	0
9f34bbb6d25bc228153120252fea4ae1	Dinoflagellata	0	6	0	0	0
7254fd18b86b6b9536284f1a6bb62b3a	Dinoflagellata	0	6	0	0	0
0ca6d037a2e4a9cb7b599337715ee234	Dinoflagellata	0	6	0	0	0
1ea2bcdc2f211f486341fe8fabf06c74	Dinoflagellata	0	6	0	0	0
bccd46d260fe1633d630f87abb2eab30	Dinoflagellata	0	6	0	0	0
4ca18577bf2581796c0a1c989ef58120	Chlorophyta	0	6	0	0	0
13f5cb02247756d98bb32f80c6efae8b	Dinoflagellata	0	6	0	0	0
10df7af41261e9bd59ef8e1103be52b6	Fungi	0	6	0	0	0
7433d644d521405dbcb654499212f601	Dinoflagellata	0	0	6	0	0
4a19dfb7d29cf762b62b1f8c27b6db3f	NA	0	0	6	0	0
3ebeb89219fc00b52783d4bc2d327b79	Ochrophyta	0	0	6	0	0

6083640809e42d178eca60113c63e96e	Dinoflagellata	0	0	6	0	0
287ab4f8ad11d2188a0c57359dd7a79a	Stramenopiles_X	0	0	6	0	0
41e2403d99f1dcf772e7f2d25376f79c	Metazoa	0	0	6	0	0
6870af3dc0518e9d888c9972a764c6fa	Metazoa	0	0	6	0	0
9e1a3a696fbdd4e776e6965991692367	Fungi	0	0	6	0	0
733d9aab0484324ee596a3accc25a4b3	NA	0	0	6	0	0
ce5f20251a7b3669cacb711c6172c2a2	Dinoflagellata	0	0	6	0	0
e09a89ffdd76ced437e61851d5102831	Dinoflagellata	0	0	6	0	0
739bcf582703c5d80161e27c242ede3f	Ochrophyta	0	0	6	0	0
1782dc236f59f5483291c6681bd991a2	Dinoflagellata	0	0	6	0	0
b0fabfef1cfb583a1641c9e8ef1979a5	Metazoa	0	0	6	0	0
a3549847fee78641d05e0c6a6417e2fd	Cryptophyta	0	0	6	0	0
74ca33141e995f09c0db05b4fdc3dfe6	Dinoflagellata	0	0	6	0	0
dc90a6c9cf4d912afed1acf692f9028f	Ochrophyta	0	0	0	6	0
396dac999e906291854034bfcdbc9c62	Dinoflagellata	0	0	0	6	0
a86ba41c8b68360eed397c1ef220c5aa	Fungi	0	0	0	6	0
2da7f63407f970053c1e3b6706c6aece	Cercozoa	0	0	0	6	0
9ea098f5bc30eb2bac44d0a70f221ee5	Dinoflagellata	0	0	0	6	0
c826f80af66e792e1b2f6992f08b2bda	Cryptophyta	0	0	0	6	0
ae4be4bee0de5c07cd29ebe9f207582f	NA	0	0	0	6	0
eb0881256391a82cb2e6f609996adfff	Dinoflagellata	0	0	0	6	0
1b938a2f87ccdaaba9e5f8374179c7eb	Metazoa	0	0	0	6	0
cee1280e7bd2c8468b7d816142923fe1	Chlorophyta	0	0	0	6	0
644832da9c8f172b17606d18136630f8	Dinoflagellata	0	0	0	6	0
4438ea1331387be0aa53b5eb8a713e7b	Dinoflagellata	0	0	0	6	0
3756a7f01635ebb4b902b184d77c5e59	Dinoflagellata	0	0	0	6	0
a6556d5a22224ed98549f0025df91eed	NA	0	0	0	6	0
0bd6fbc3fc03182a3ef20cd9b0815a2f	Metazoa	0	0	0	6	0
9b524f3ccf399de41f7b7d8d7a3224e0	NA	0	0	0	6	0
07cc0096d53855f2ba4f8ab44e480b3c	Dinoflagellata	0	0	0	6	0
6874e327401512fdba24b4721df3bbb9	Dinoflagellata	0	0	0	6	0
c5a18e12af517f8f895a6a592b7239a4	Metazoa	0	0	0	6	0
5c961f36f44c5ab3e9a9ace13c85caa2	Metazoa	0	0	0	6	0
8b6f2cb9da7599c24dc60bba42de6f46	Ochrophyta	0	0	0	6	0
bde2a25c516dadc5b32bb6f8fa0390d9	Dinoflagellata	0	0	0	6	0
6049a165b28dc4c7eb6898637c9cc3b0	Dinoflagellata	0	0	0	6	0
ed456f5531e0dea1cce49ac3acfbeb24	Dinoflagellata	0	0	0	6	0
a789b73ee64d8b2872b4465ce58c1353	Dinoflagellata	0	0	0	0	6
af32b491dcb4f66fd7416c568d33900d	Apusomonadidae	0	0	0	0	6
23134966295fccaacc5c6f45a9466dc5	Breviatea	0	0	0	0	6
eaeddd84c125e6f0cb1d3dfde00d7b61	Ochrophyta	0	0	0	0	6
23681ea633f1a40160159abd441dec04	Apicomplexa	0	0	0	0	6
912b44334553a52a311119fe922b9fda	Eukaryota_XX	0	0	0	0	6
217ca148196cf04309c4014f37b048b5	Fungi	0	0	0	0	6
afdf5a2e94bfce689d981402d72311a5	Dinoflagellata	0	0	0	0	6
70a1885612e725d172b2b6fef7c73f5a	NA	0	0	0	0	6

861f6412c0b9f4e342fac6add9ba1d09	Dinoflagellata	0	0	0	0	6
e4b2ee440995fb8b77b42fb3782829bd	Eukaryota_XX	0	0	0	0	6
5138703f4725512caa73ce5090dc12e9	Ochrophyta	0	0	0	0	6
437c7e992aec306b93418eb0d696bb97	Stramenopiles_X	0	0	0	0	6
1162a2ba1f97cf9a75988309f907cdc9	Ochrophyta	0	0	0	0	6
83bf9d275d96355ee67a8331d28b8764	Dinoflagellata	0	0	0	0	6
ec848158be6afc52395f13bc3736aed1	NA	0	0	0	0	6
3995640eac0a9762394bb03328877491	Stramenopiles_X	0	0	0	0	6
80009c9d1dd7a65455ba90fd03278dfd	Fungi	0	0	0	0	6
3868b714800f47f3d851dc4f7e96f1f8	NA	0	0	0	0	6
2a376972136d5cded0331e5a013aeb22	Eukaryota_XX	0	0	0	0	6
3672632325dc45df8ee1a31eb05c1e27	Ochrophyta	0	0	0	0	6
8b98598d3c89f4fd4b0db0b01bc5a4e8	Dinoflagellata	0	0	0	0	6
baf4e07c31f7da1ffde0a0a171cceea6	NA	0	0	0	0	6
1eeeb8d665b828fd4f941257c71039e1	NA	0	0	0	0	6
7072622156cb2192bc233c7295dd06dd	Dinoflagellata	0	0	0	0	6
f0e72e88c85b9db609bc6fcad823661f	Eukaryota_XX	0	0	0	0	6
9b02c6db8930e833aa424cbc22332c2b	Lobosa	0	0	0	0	6
86237e7e2bdf9287228be9a69e266fb1	Ochrophyta	0	0	0	0	6
56e523b1c13590005dc691af86e519b4	NA	0	0	0	0	6
f0adb19c55512dbfbb0c32e307fa8686	Ochrophyta	0	0	0	0	6
450b0c461cd53870392d8d13720bbf6a	Ciliophora	0	0	0	0	6
538b5b131fcb4338cee00f0de7fea198	NA	0	0	0	0	0
422dfec20291df1c91bbbdc6ef1115e9	Dinoflagellata	0	0	0	0	0
e4dfcab3f3e769668fb4d1b0aae08a5e	Fungi	0	0	0	0	0
d905250b1e5647cb574efafb39cced08	Ciliophora	0	0	0	0	0
1b8b2f0eb9d5b2af0ea1baccda2842f1	Dinoflagellata	0	0	0	0	0
6f1ae8814a834911f3974e73c638f433	Dinoflagellata	0	0	0	0	0
6a5fd1b3384f98625b21b864a852f193	Metazoa	0	0	0	0	0
7c6676117ac21f4d59eaa7b768c1a6d1	Ciliophora	0	0	0	0	0
88c6f5b1e13da23ed503814b62b34170	NA	0	0	0	0	0
be736223b7337789ed88b753307a122c	Ciliophora	0	0	0	0	0
2654ab72a343710fb7f50dcdf0a46aec	Ochrophyta	0	0	0	0	0
d924ef28f2ffc30f50831e26c9f6684d	Chlorophyta	0	0	0	0	0
735a4c861c0cc20f7a499fa899587eab	Dinoflagellata	0	0	0	0	0
77e64f3df46b1e4ec8d7f6b78e50b30f	NA	0	0	0	0	0
d61b161bd752e0f8d6a4aaa92a701c63	Telonemia	0	0	0	0	0
e43003b2cdb9dbf769c491592caff8af	NA	0	0	0	0	0
64f3bbcd58a3464c7d888f55bf1bd263	Dinoflagellata	5	0	0	0	0
a635c2d51ff886c01274eac4d0581f61	Chlorophyta	5	0	0	0	0
5cdb6684b19c814e3073eca930116ec4	Ochrophyta	5	0	0	0	0
29c6ef1af96a29b92c40a114b325c2d3	Ochrophyta	5	0	0	0	0
08d18e762205cd1cfd44739a699bce77	Stramenopiles_X	5	0	0	0	0
c4fd5805f3c716fe8f9c124290edec8a	Ochrophyta	5	0	0	0	0
1a61a4085a738a91827f5e5e0598405f	Metazoa	5	0	0	0	0
f5d580d8c01bdbc1141519eb7b8ad880	Metazoa	5	0	0	0	0

42336eb8b1a71ea6c49d1e8c24639591	Dinoflagellata	5	0	0	0	0
06598495a58f9e7feac1d5ed5a651f4e	Metazoa	5	0	0	0	0
07d409f5ab9616b88e27de38fa38e238	Ochrophyta	5	0	0	0	0
404b05f6e741981f280410e2f2d5dc21	Dinoflagellata	5	0	0	0	0
b4380836c4eeb574fe26d94c0bdd198a	Metazoa	5	0	0	0	0
2f7faccf2565f49106aba0cc16426cf2	Dinoflagellata	5	0	0	0	0
06e80273c4b75f0ef00ec2d3b263664b	Ochrophyta	5	0	0	0	0
586b4d392245c6013e752cbe9ff905a9	Lobosa	5	0	0	0	0
099d3d0ca1415183a8dfb5600249a21d	Ochrophyta	5	0	0	0	0
883fe87c379dd2b5db3c9a61906fdf9f	NA	5	0	0	0	0
80e52a3e979a92abf30af0b422c8bb3a	Eukaryota_XX	5	0	0	0	0
42b75cf84cfd71721b27c4ea670f25a1	Ochrophyta	5	0	0	0	0
7e6aa3dc6b0860dd2caf77c72598df66	Dinoflagellata	5	0	0	0	0
ca8954fa1ce45c8d26fd28c11aad461f	Dinoflagellata	5	0	0	0	0
1c71e2cceca53c21019d1d05703e8f7b	Dinoflagellata	5	0	0	0	0
236ab6c0a2c94afbf7b0b87039a1ef75	Eukaryota_XX	5	0	0	0	0
da872024b094c8511470367ce587af84	Dinoflagellata	5	0	0	0	0
34173193a7bb80560bf0a09072f627e3	Ochrophyta	5	0	0	0	0
79562db081549764be5bcb967bb56695	NA	0	5	0	0	0
09fe526f959c42a6dad9f09b49382d66	Lobosa	0	5	0	0	0
ebb1ad9cf0934a7c9c44581c8b0affa3	NA	0	5	0	0	0
ae10c2f6d3885dbd82aff5b22c2e0fc8	NA	0	5	0	0	0
f9f1faf101613797e274332dfb47b928	Eukaryota_XX	0	5	0	0	0
50348526cbaf86ad7820cd3f1033d520	Ochrophyta	0	5	0	0	0
9f6db4d71d0ff06933fa8d583533edb2	Ciliophora	0	5	0	0	0
034f4b753f6a360c7a8dc1a152bd30d6	Dinoflagellata	0	5	0	0	0
f43ed7cfb89b368fcb8c4600615096be	Eukaryota_XX	0	5	0	0	0
edca1356f220e8a328e644b129ad51bd	Haptophyta	0	5	0	0	0
5a573576a3e069b6308e3b439fec20cb	Chlorophyta	0	5	0	0	0
f91e052667813c6b128fc45f024cbd0b	Ochrophyta	0	5	0	0	0
1d384034d3ec82dccfe6301b68809e40	Eukaryota_XX	0	5	0	0	0
283cc68b65503c6540fce3992456ba3c	NA	0	5	0	0	0
0ee558d16f1327a77c1548ff63a7509a	Eukaryota_XX	0	5	0	0	0
6cfa61b5758d05576cbf8d23187518af	NA	0	5	0	0	0
e40607913d36954150d0e32ebce285a5	Eukaryota_XX	0	5	0	0	0
3d1bb8b2aa2c7774ec3fb279534d1559	NA	0	5	0	0	0
0b95fa7825822dc8db57d8ca22fd6491	Metamonada	0	5	0	0	0
95ab6c3dc8f3fc43aea6e9b3da1faf23	Dinoflagellata	0	5	0	0	0
d76c7b8440e28191dd948b44aa366dcb	Ciliophora	0	5	0	0	0
785b3a18cc303e36890e84498be8d4fd	Dinoflagellata	0	5	0	0	0
94f8ee5a5301a7634e6738c7af2a5aeb	Dinoflagellata	0	5	0	0	0
0edef2e986dc83a6acd85f0fcbec193f	Picozoa	0	5	0	0	0
f1491f710e7b8725c72cf5b3fadd5005	Dinoflagellata	0	5	0	0	0
e2fa53e86a6decac112aac2eba99a28b	Mesomycetozoa	0	5	0	0	0
46138dffc819ba9abbde56810b6cd566	NA	0	0	5	0	0
54ed5c07e552a269dd610bb265a3f053	NA	0	0	5	0	0

5bdcc2f1f28871c0e11f0562ea8c5a64	Ochrophyta	0	0	5	0	0
1e3791e53afcbf0a39f6c08be75d1aca	Apusomonadidae	0	0	5	0	0
2e1555d8d6e3ee6fc8d36b7c153fbdb6	NA	0	0	5	0	0
0367c90728d61e276465019a05e32a25	Dinoflagellata	0	0	5	0	0
382ff2b85f837596e846da8630f35c20	Ochrophyta	0	0	5	0	0
227e485430a75f05ff113c0ee328e356	NA	0	0	5	0	0
c540b2109dfb9e1e64e6175692df5be4	Metazoa	0	0	5	0	0
9c868bc5870e653e5bc08c9c28ecfa57	Dinoflagellata	0	0	5	0	0
d5e7f448780e18edebc73e7afd6b9dc1	NA	0	0	5	0	0
e0fc28af205a172319d803fc84423ab3	NA	0	0	5	0	0
3d5302fee107bc07670634b5b1da2981	Ochrophyta	0	0	5	0	0
4f7dfd48e1eec808f2c3ecdd4eaec58e	Eukaryota_XX	0	0	5	0	0
6e222432b03ed77a57160d081bcbd952	Dinoflagellata	0	0	5	0	0
6de5e78ec2f5ca61da38071c94dd3d7f	NA	0	0	5	0	0
e74562d782793493b690bc0b335a3801	NA	0	0	5	0	0
6c2e1b8aa982b4b2c510a86021c9956b	Dinoflagellata	0	0	5	0	0
e270614f6cd445ecb09ffbc238645184	Ochrophyta	0	0	2	3	0
19fcaf12b735bbd99162a82c548bc657	NA	0	0	0	5	0
4bda6c5824936477ce33bc3c86ca4b7e	Dinoflagellata	0	0	0	5	0
2f164269f86bd31ae732a3726abfa09f	Dinoflagellata	0	0	0	5	0
4351d79dd21a8a2bb86a0fe8a27bb9d5	Dinoflagellata	0	0	0	5	0
80e564212d725c9b0baf432c6afbfe9e	Haptophyta	0	0	0	5	0
0b06d6c40fda30a0e1ef8be34b67876c	Dinoflagellata	0	0	0	5	0
b33453696521ee40ba7c740f812ea50c	Dinoflagellata	0	0	0	5	0
93bb59e18ea78cfb6013531a40d5ed29	Dinoflagellata	0	0	0	5	0
841ac36e58acf22c3c57fe633ca9eb79	Ciliophora	0	0	0	5	0
5fdcdbcdcc9649ac63d5fdeef781d9ab	Dinoflagellata	0	0	0	5	0
6857e26c34af7db6094fe0b76f829887	Dinoflagellata	0	0	0	5	0
14ceb1ac57f89d8ec66974083e46481d	Dinoflagellata	0	0	0	5	0
4fbae2d74605b7a360f1462e1b99c131	Eukaryota_XX	0	0	0	5	0
357611dba2d9fdf65229b82d589035c0	Dinoflagellata	0	0	0	5	0
2e7e801c3500d2146871b1a7871335b6	Metazoa	0	0	0	5	0
7489436c63a0db9828a628f995dad462	Dinoflagellata	0	0	0	5	0
b96a454038cd205643fd806a24f3b05c	Dinoflagellata	0	0	0	5	0
ee5a7e68df8932e40e07306235c16c23	Mesomycetozoa	0	0	0	5	0
7f5a73191164ecfd4096b82798072f5f	NA	0	0	0	5	0
06346f6334d80a223f2ae937c21535b2	Picozoa	0	0	0	5	0
fca2b4007a8a41d566a4aba695e4682b	Chlorophyta	0	0	0	5	0
137cd91087964b932bcd08d20ff71e17	Dinoflagellata	0	0	0	5	0
7dd8d30c7ab160ef8274bcc593b3be80	Dinoflagellata	0	0	0	5	0
25f349e1fab3c846a13b91eed35c927b	Chlorophyta	0	0	0	5	0
7a1bdcfa5fbcc052afa060d1568d0124	Dinoflagellata	0	0	0	5	0
c33df10e57c1a5184cc7623941388107	NA	0	0	0	5	0
3bf8991360e91735b00402d0d0e40cd4	Dinoflagellata	0	0	0	5	0
3043ec8c61f6073abca6694e34dd5eee	Dinoflagellata	0	0	0	5	0
7ba39fd3f4a3aa5139f877999181c389	NA	0	0	0	5	0

7ab855c8b73d7f8c5c82d0c12407c1ab	NA	0	0	0	5	0
0eec8d024bed07d08177c090a9a06e08	Rhodophyta	0	0	0	0	5
7238a89ff24e8440467eab48631ed117	Stramenopiles_X	0	0	0	0	5
3308741e1585bf15032aa3e61924067c	Amoebozoa_X	0	0	0	0	5
ccdb4722348f4cd709a407159241b09c	Fungi	0	0	0	0	5
9ee8558e2c948c587811dc44d0b472b0	Ochrophyta	0	0	0	0	5
2967efeae9db12279a4c58f1e3805133	Ochrophyta	0	0	0	0	5
d705cd023827cf11b5482fc96a1ccd7d	Dinoflagellata	0	0	0	0	5
d7f17f01a165be767a5015657c12f330	NA	0	0	0	0	5
71fec2f22b4c22a4dfacf2501812f5e0	Eukaryota_XX	0	0	0	0	5
05b0ec42587ceada882fe71d6cc2c9ea	Eukaryota_XX	0	0	0	0	5
7f52b57a3fa8c723aff64d92cd6dc5ca	NA	0	0	0	0	5
e5baa6b1e601e1df58878a7a9efc303b	Eukaryota_XX	0	0	0	0	5
d521de6664c0c5660ac6df1be417bdd6	Choanoflagellida	0	0	0	0	5
0bb2aaedfc142ac54062969878dc0b08	Ochrophyta	0	0	0	0	5
70c3eea0919067a9185d1371b275c579	NA	0	0	0	0	5
63154806b91dd55f5ff0c802e595b17e	Fungi	0	0	0	0	5
d8f6f5fed82123acc6426e713c21768d	Fungi	0	0	0	0	5
34ae418a179881e9527f80fc2eb57456	Dinoflagellata	0	0	0	0	5
9e4ca9dd466a18d94136bbfeed19071c	Metamonada	0	0	0	0	5
19ef5dcfb8f09354d17e20203f8f1e3d	NA	0	0	0	0	5
1c485b95586509b087763aeda6941a9d	Eukaryota_XX	0	0	0	0	5
b756f4ee3f3b31ff6c6b108248673726	NA	0	0	0	0	5
f89cb93eadb2fd58eef40c570fd5240e	Fungi	0	0	0	0	5
02c5145da7b4209dc17884761eb9822f	Ciliophora	0	0	0	0	5
ebfe507e8f468d015fff2f337638d0e6	Dinoflagellata	0	0	0	0	5
00c2c2635a8fd15492762c2131f56e5d	Fungi	0	0	0	0	5
0cacf3b4a25b791aeb51e4dfed130102	NA	0	0	0	0	5
2ee4942ae0c4991fb9e3e563ebc44b3a	Ochrophyta	0	0	0	0	5
49b411aa7347d4dfb92ff7305247c555	NA	0	0	0	0	5
fd7e0888665eec1797ede2ca543b1e25	Dinoflagellata	0	0	0	0	5
7a84dbceaacc4052b0210f448d6c2c71	NA	0	0	0	0	5
c11d967e83fd3c2406b9f27c88dbbc93	Apicomplexa	0	0	0	0	5
ba39e7c393dcd1337df88a525c7cab15	Fungi	0	0	0	0	5
c86599903f9e5ea075631e64bb23c939	NA	0	0	0	0	5
81ffa9596b482d8773ffdb1c3465d17e	Hilomonadea	0	0	0	0	5
a983f7da7e9ccaaf1f711e01b416f686	NA	0	0	0	0	5
ab3f6b6290d0ff7a6a139d6e08a4684d	Eukaryota_XX	0	0	0	0	5
1690da72286db1621e1a38139a85c196	Dinoflagellata	0	0	0	0	5
ec89b7fa7e518c7fc6342ebc94766111	Dinoflagellata	0	0	0	0	5
afc4e2a42b149d607a81581190cda109	NA	0	0	0	0	5
98689937ce497cb09127fbd65dd1a28e	Eukaryota_XX	0	0	0	0	5
6999c1348b4cb41b635e3ca48a82f88f	Ochrophyta	0	0	0	0	5
40b90ddfe2f6bf5bb7ba2740929cb2de	Dinoflagellata	0	0	0	0	5
f81b2c4adb072736f15d7233c684c09b	Ochrophyta	0	0	0	0	5
d33e4d2f9db6713c4aaf112b78756fac	Ciliophora	0	0	0	0	5

7fb19dee6f4749f5debfe98f12583389	NA	0	0	0	0	5
035833f94f941e25f71bc79b8f85ac6b	Metamonada	0	0	0	0	5
3af39a257c2dbc7e8625dab36a0bbf7f	Ochrophyta	0	0	0	0	5
f23ab82665582e3668cd003925c0519c	Ochrophyta	0	0	0	0	5
8ccb895c005919b5d4bc45dbc259e5a3	Dinoflagellata	0	0	0	0	5
56a020d976470897e867c942f2f827f8	NA	0	0	0	0	5
2f7e1e1f4078b3118000f1227f290856	Dinoflagellata	0	0	0	0	0
4257ad814611b7683f92cdabed4c9862	Eukaryota_XX	0	0	0	0	0
478119f1cf051281caabe18bdf7efcde	Dinoflagellata	0	0	0	0	0
7836e50da253cd8ca061dbd47c17cc16	Fungi	0	0	0	0	0
759f3997df926a65f2d3f1d526358033	NA	0	0	0	0	0
35b3fed6840bee85ac4c97ac49fb29ee	NA	0	0	0	0	0
1737ca59847988b3711ccc1b2af3617b	Dinoflagellata	0	0	0	0	0
67bf6222fc7a3dbf2e8b36a2ebf0939b	NA	0	0	0	0	0
2a8fdff97a0b33ad89a022241fbf21e4	Metazoa	0	0	0	0	0
910c3ee01f5872132180f1aae75c37d5	Dinoflagellata	0	0	0	0	0
a0362cc36d9c383f3cc48cf2c64e3062	Ciliophora	0	0	0	0	0
c213c99a0b88477b1ee0eca4460a36ba	Ochrophyta	0	0	0	0	0
e3131f1ac26f75753c3da9ee5bc22fcc	Stramenopiles_X	0	0	0	0	0
b68a899464c16f921ce18a771e5b13dc	Dinoflagellata	0	0	0	0	0
d708ed7cca311401cdccb02ee5eac24c	Chlorophyta	0	0	0	0	0
32132274e44f1226c620b6f490c7d6eb	NA	0	0	0	0	0
36a4ca8e76e8d751344e10ff5973d9f5	Ochrophyta	0	0	0	0	0
20506f6a615cf404c352d2336fbfad33	Ochrophyta	0	0	0	0	0
04d6848885573c5748ce425738a99e31	Dinoflagellata	0	0	0	0	0
99daa5890605de91b39fc551134b0fbe	Eukaryota_XX	0	0	0	0	0
b860681a09fb75387271368946c45131	Eukaryota_XX	0	0	0	0	0
ad593ccc10b8bc53d938eba9de61d2f3	Dinoflagellata	0	0	0	0	0
e62f429a6880ddbf8192216cc518d5b3	Eukaryota_XX	4	0	0	0	0
663930e61c272939dfcf9c44bef9a83c	NA	4	0	0	0	0
ded6d696af692566c8ef58a02ff9bf9d	NA	4	0	0	0	0
179e7b247b815e0f9c2e9d37436df994	NA	4	0	0	0	0
0df15a1d40ca47fbd729b9e935a468c4	Dinoflagellata	4	0	0	0	0
3a7a4c35d7eaad7889e34f9fa87d7ee7	Eukaryota_XX	4	0	0	0	0
812c7657a159935ab404afa04b47d6a1	Eukaryota_XX	4	0	0	0	0
efa0ce72635f2796c7f82653bad9920b	NA	4	0	0	0	0
fe9aaaa2f8cee48292d91e03ba733d8b	Dinoflagellata	4	0	0	0	0
ea28a74dd5a9356e7252d3dd4c50fe06	Eukaryota_XX	4	0	0	0	0
92d01ab8eb8fa973c05a4be1c3920425	Ochrophyta	4	0	0	0	0
d68ceefa51d120e813c8c98d78ef67e6	Dinoflagellata	4	0	0	0	0
9c72ba8947f38b1b7b55275cdab3aaad	Ochrophyta	4	0	0	0	0
f96e5bc819c894ade4250e56191188d4	Dinoflagellata	4	0	0	0	0
a11728b08faa9f1ad0ef1dba0a999540	Eukaryota_XX	4	0	0	0	0
f3fdf6b4fe2697647285568d21f73c9a	Ochrophyta	4	0	0	0	0
03dead33d0eeae737aaa1a0e8aaac4b8	Eukaryota_XX	4	0	0	0	0
c443074e65074753c6f48c93ce929aca	NA	4	0	0	0	0

abd3fd53fae6bf7afb5f0bb336c038f1	Dinoflagellata	4	0	0	0	0
104dca01d8bb31543fc99ee5524ddd1a	Metazoa	4	0	0	0	0
00617ddf159fab8e543a8ffe69ed742b	Dinoflagellata	4	0	0	0	0
50111c4e5423d8ecfce6a5d7287d4cde	Metazoa	4	0	0	0	0
f9ee9796ab41290a81d2037b2e0af152	Ochrophyta	4	0	0	0	0
dd160bb0c22f2362fb5afc38bd97a5ab	Metazoa	4	0	0	0	0
b8ffe6e68f2257483f5aaeae3e8a5671	Eukaryota_XX	4	0	0	0	0
92bd157c63802177b728bb84a561c533	NA	4	0	0	0	0
f5de65ff78eb3c51e4384f448ec0c1ab	NA	4	0	0	0	0
a57bc25ccf8c30fde82987f91bbd1a6e	Dinoflagellata	4	0	0	0	0
0d43e434db2d2331ef29c4ed95c553b1	Ciliophora	4	0	0	0	0
7b88b1eb439aecce098aec935fad5277	Ochrophyta	2	2	0	0	0
83e19e10482ccd93f93b79cdf601d749	NA	0	4	0	0	0
d21bc2497fb1a0e72d2b3b26a84cdb86	Fungi	0	4	0	0	0
2acc0f6e15ff5f76380c66705292be1e	Cryptophyta	0	4	0	0	0
3105a6636ba51c8e988a227f3434529c	Dinoflagellata	0	4	0	0	0
38665a631c165de69c66527393856711	Eukaryota_XX	0	4	0	0	0
f37e4478c55cce603d389cb6cebd657a	Eukaryota_XX	0	4	0	0	0
c9aac5fa85e7be49c1cf9db7e00f02fb	Dinoflagellata	0	4	0	0	0
daddbf0b5ee7ba592025edf4b834f03a	NA	0	4	0	0	0
6e21754db9365e74d27e560731cba372	Eukaryota_XX	0	4	0	0	0
bcd6ba5974f47816d8d83b7704a027ff	NA	0	4	0	0	0
6c91a08430f89ebe222995965f527c45	Fungi	0	4	0	0	0
513d34a369e99957e5ac55f75475a7e9	Dinoflagellata	0	4	0	0	0
e9667f751bbe046480e50e0f106ffcf2	NA	0	4	0	0	0
4906056b1a654deca932edd5cbb72a4a	Eukaryota_XX	0	4	0	0	0
b512fca70cf9e85a2a7ebe42b4b84e56	NA	0	4	0	0	0
a9d90d939f3abb38d846444e890e854a	NA	0	4	0	0	0
71c1c3b3d6c63f4bcd94e8b40e33dbe8	NA	0	4	0	0	0
47e0989139e817a8e727852722ad33e2	Dinoflagellata	0	4	0	0	0
a7976da626b53c9b1cd4ccea16fcf43d	Dinoflagellata	0	4	0	0	0
f6a69caaaf44c8d4e858a13cf5493cd1	NA	0	4	0	0	0
55b0f4ae04fdc7e3a9293a3a7bfec029	Dinoflagellata	0	4	0	0	0
705d39a1b24bd64cb01d27ba3a1ee2f9	Ciliophora	0	4	0	0	0
3c4d09bd1220b4ab4434d88ab1637d9b	Eukaryota_XX	0	4	0	0	0
2c2c1c5689923179b9890cadc736e688	NA	0	4	0	0	0
8214d1c4c6008d7c5392c067b6115a50	NA	0	4	0	0	0
bc643d22b93fced9c2458cf66fdf7d27	Chlorophyta	0	4	0	0	0
80cb9f4cf0915a7e382a454a0ee0396a	Stramenopiles_X	0	4	0	0	0
a73df56a7760524ab4a131c955c8a28a	Foraminifera	0	4	0	0	0
7bb203fea10dada2974815461f2a77dc	Eukaryota_XX	0	4	0	0	0
bf9447a93140caa306c74c4d5ae45b60	Stramenopiles_X	0	4	0	0	0
e80016cb2a6d1d007c2a5f42e49daf10	Dinoflagellata	0	4	0	0	0
97872e3d5dfa46df903dc98b47c97ad7	NA	0	4	0	0	0
093ba71bbdc172880c7ad91553a09eb2	Dinoflagellata	0	4	0	0	0
d0a6e2be66643d4c515eb688320255fe	Dinoflagellata	0	4	0	0	0

6da94bba15aa56e53d67c27d6b99469f	Metazoa	0	4	0	0	0
d256ac452655a95cc65ab97d7ea6edb5	Eukaryota_XX	0	4	0	0	0
3a69b18b7ce4bcf599f8ba919441201a	Cryptophyta	0	4	0	0	0
559866c506737a9d090f9441527bb59e	NA	0	4	0	0	0
3649c6f7ef6caf301240516f8bcaaf36	NA	0	4	0	0	0
85da92e2ada513b21345c62e614580c6	Eukaryota_XX	0	4	0	0	0
3218822d17e42c8e3fa6bbae03c0c41d	Ochrophyta	0	4	0	0	0
c05563185246e6039cbaba88419f19c9	NA	0	4	0	0	0
edc7d181d563efbe864ccb466ccc5230	Eukaryota_XX	0	4	0	0	0
81442951181b73452fd5c7f327d5dec5	Dinoflagellata	0	4	0	0	0
20b9d86d9d2f801b8d71d36460c577e5	NA	0	4	0	0	0
8c458f4d27dd9228242fd7c78d2101ea	Dinoflagellata	0	4	0	0	0
a91132f8d916737a4a2ed1248475b97e	Dinoflagellata	0	4	0	0	0
6546a56be0c5a8276531bc8b93d7c9a8	Dinoflagellata	0	4	0	0	0
5a04c920ebaed8f23ba2e2082801e5ff	Ochrophyta	0	4	0	0	0
04df393a64968dd6e9f3b420b2e31ac0	NA	0	4	0	0	0
47af8459380099d0c9a6c580c5d22e24	Dinoflagellata	0	4	0	0	0
94072a295fa07d7225be4e95cdf97dd6	Eukaryota_XX	0	2	0	0	2
df9a8fd5cd2f14199d7e2ba68d10b523	Dinoflagellata	0	0	4	0	0
7e92e08701ce04c8a8bd7b3d8a437191	Ochrophyta	0	0	4	0	0
abadec9f8b209221067874be2d0bf58d	Eukaryota_XX	0	0	4	0	0
c37552c80afe17bcd2c6cc688b0de1db	Eukaryota_XX	0	0	4	0	0
082dac5fb05dc4fc8f196c33d8bc4463	NA	0	0	4	0	0
2980682ee989dc8a9cd7ccde2912a845	Dinoflagellata	0	0	4	0	0
6f46df06d3c5c5469d2f7dea19f4dce3	Eukaryota_XX	0	0	4	0	0
591a47a73fc1cc4a59aea27eb8b2cb5d	Eukaryota_XX	0	0	4	0	0
e1b91ae6a3522ead43a48bdcc312b43c	NA	0	0	4	0	0
f0c2770a66c5c22ec50222b8e8566ed1	Ochrophyta	0	0	4	0	0
278ab49b01d1e6720041781ef6d3498f	NA	0	0	4	0	0
360c2817052461f4aa708969181e1953	NA	0	0	4	0	0
69065ca4d256e344de934221ebfd81e6	Ochrophyta	0	0	4	0	0
bbab35030e7bfd130b391255deece450	NA	0	0	4	0	0
9618f78ba546ccf19244a605d2c89334	Metazoa	0	0	4	0	0
a8d632aa41b7bcc197c1c7e8def597d6	Eukaryota_XX	0	0	4	0	0
de164dcbcd04ea946485de71951ec154	Dinoflagellata	0	0	4	0	0
61c4859fda5bf832cf519cda9327a869	Eukaryota_XX	0	0	4	0	0
73d760d9e6050c409acdd22d84e18c8f	Dinoflagellata	0	0	4	0	0
1be137d1e7608356510fe4ffd34479a5	NA	0	0	4	0	0
2c687b8409a537dc08c78d621414f640	NA	0	0	4	0	0
536ddf25c9a1b2d05a47f8aa8609ad7e	NA	0	0	4	0	0
9919f965f0118967e1af21d467d6e037	Dinoflagellata	0	0	4	0	0
8f4b3ffabee4dee3664c21c41ddaf5b7	Ochrophyta	0	0	4	0	0
505e9bde76c164cca3e5b61d74cd8394	Chlorophyta	0	0	4	0	0
157354a8641f6e31701175e47488a255	Dinoflagellata	0	0	4	0	0
ddc7ee6c0e9585e19bc3547c40abbeea	NA	0	0	4	0	0
8014461b4a1fb2245dd5d9f543638e5a	Eukaryota_XX	0	0	4	0	0

c673f21ad059022995a9a3cdd65eb28b	Dinoflagellata	0	0	4	0	0
a71e1024babc70cae3e6fdf3dc038039	Eukaryota_XX	0	0	4	0	0
cb7cb5434d3f54d8c1976222bf45cdfe	Ochrophyta	0	0	4	0	0
d833b4d00f3c675e90b36495e998ae0d	Stramenopiles_X	0	0	4	0	0
569b45436910cdb2cc390f6d71974876	Ciliophora	0	0	4	0	0
4cd9c1a0a1e4af43c0e141dc3f028546	NA	0	0	4	0	0
117890740b70533e34b8fa8bbd7d658b	NA	0	0	4	0	0
57874de590e7b7a265e13a14cbca5b92	Metazoa	0	0	4	0	0
d300ffd3556f981b73992211cffb2a34	Dinoflagellata	0	0	4	0	0
15e58d4919780196258172852a19996e	Eukaryota_XX	0	0	4	0	0
da890018101e2c9ac97aa95bd36ff51c	Foraminifera	0	0	4	0	0
9f203495f6f14091ce0474c47b08d45d	Dinoflagellata	0	0	4	0	0
25fa332cbc790c54d28ffb448bc13790	Dinoflagellata	0	0	4	0	0
1204e33509a1ee04f30523583f3c0bc9	Eukaryota_XX	0	0	4	0	0
a32c49a5c327198f2aef24f6c2a6cfdf	Ciliophora	0	0	4	0	0
c39401aab651646a879b0f7f199c6634	Dinoflagellata	0	0	0	4	0
e4d90c1c9d4a379d20218fac21ba9d88	NA	0	0	0	4	0
950aef235ffb7e7060004d421b107d70	Telonemia	0	0	0	4	0
a8dd507653359e827528a9104e7eea3a	Ciliophora	0	0	0	4	0
8d8a14eec7ff82a9ca63a4a1e7a75671	Dinoflagellata	0	0	0	4	0
b6dd2fb11e7010b535584faa9cdf311f	Ciliophora	0	0	0	4	0
219a3c068f33be3104c721309a7bce9b	NA	0	0	0	4	0
c9ecf444c06a5245e2f2ce50bb699408	Dinoflagellata	0	0	0	4	0
958e7cbcd5238fcc76955953072441d6	Dinoflagellata	0	0	0	4	0
d4b01902b32e24922c33cab5bfb6ecb5	Metazoa	0	0	0	4	0
2bec4a44cc85f38ef9522dc9c6c9bf3a	Cryptophyta	0	0	0	4	0
ce622de37da61c67ec7eeafe579b82d8	Metazoa	0	0	0	4	0
63c0ff3ed0fccfc32ea2527abc2f9e0a	Fungi	0	0	0	4	0
6532184cf2c640d7ad5de6565c3eb30f	Dinoflagellata	0	0	0	4	0
a3b0b2fa5a6abf0245008f45f85ace9a	Picozoa	0	0	0	4	0
b0aa7202f8870ec3498b5206c4cb3c00	Dinoflagellata	0	0	0	4	0
6f178370468e7b7883a0c87ac66440d0	Dinoflagellata	0	0	0	4	0
f620284f8475fca69ae6f465f9a2ba5b	Dinoflagellata	0	0	0	4	0
1d41580d6764887ec45d6809b7753fb0	Picozoa	0	0	0	4	0
e682eb11202d0b21cdfa540ff9efe072	Ochrophyta	0	0	0	4	0
b98e5a51e544b1064f1dfe815b9e66c2	Dinoflagellata	0	0	0	4	0
3c7b87d773bac13c7a561b2643696238	NA	0	0	0	4	0
293b41abd003645bb707020a5578012a	Dinoflagellata	0	0	0	4	0
1e54b3d8b70b5324ac5a2032dabd9788	Dinoflagellata	0	0	0	4	0
37bf569a9556e8b4d6960c3ddbd649db	NA	0	0	0	4	0
5a2292cb1c30929dfc2c9053e84ceceb	Stramenopiles_X	0	0	0	4	0
9f4a11557196185f36d62ea1f15995e7	Dinoflagellata	0	0	0	4	0
4a6de5bd8c3e5b38404f8a030ab21045	Dinoflagellata	0	0	0	4	0
130f84ba6588bd4d41e0fcf3bfdb0cc6	Dinoflagellata	0	0	0	4	0
609349a64f2319220808b163aad22946	Dinoflagellata	0	0	0	4	0
8d43f9aec4d7e040ac177d8260a24f4b	Ochrophyta	0	0	0	4	0

7b2a360b0fd7d04e6bd1d9a6a0a6a218	Chlorophyta	0	0	0	4	0
4f59553ea0ec7819ad395bcdf5118788	Dinoflagellata	0	0	0	4	0
f4f7aba4b5a0b8f437c1ed3c19d1e71d	NA	0	0	0	4	0
699fd35252a5d13d3b2a759d295571f5	Ochrophyta	0	0	0	4	0
c94ce6d34c382d0850c73ab05f286d2c	Dinoflagellata	0	0	0	4	0
bda26145fbc03d67ff91d1c95e6132d4	Metazoa	0	0	0	4	0
de3536f0469e81a783b47171e1a5a984	Katablepharidophyta	0	0	0	4	0
58d2498485617ff1074b65d21049ab5a	Ochrophyta	0	0	0	4	0
fa5751aef369d5cd7cc0116fdd1c44cc	Dinoflagellata	0	0	0	4	0
8647f959b58877253bafac6b81424bf8	Metazoa	0	0	0	4	0
560265c795e946bf43672c138a85d6c4	Ochrophyta	0	0	0	4	0
38d611809452a08a5e21c6f200127727	Dinoflagellata	0	0	0	4	0
55dbc5eaff86645bf921ac67e129ad25	NA	0	0	0	4	0
38b8c8f4effbded6a0fd83c58d6e93d1	Dinoflagellata	0	0	0	4	0
29482865be5732e7e75a698f103ec028	Ochrophyta	0	0	0	4	0
b5493ea3cdc357dfc4015ae5051b750e	Metamonada	0	0	0	4	0
ba86b1c9e13182897e434ee2dd4da572	NA	0	0	0	4	0
b86db8015cd028d7294bcc2545152e12	NA	0	0	0	4	0
ad118b9fe41279e26fcad81a1af1fecf	Dinoflagellata	0	0	0	4	0
63209679e748976b2d34b04983bdf43f	Eukaryota_XX	0	0	0	4	0
f488e3123c660b6c54da5afd44978867	Dinoflagellata	0	0	0	4	0
051bb9d8c1c580800070c42fa52c5ae9	Ciliophora	0	0	0	4	0
95f7420866644aead6e163d71349d6b4	Dinoflagellata	0	0	0	4	0
bd7bce647b5eb43ddba72239023e8034	Dinoflagellata	0	0	0	4	0
9f0243ef32ed61cdd264754854ae26d1	Dinoflagellata	0	0	0	4	0
8d8ae9f2bac2ee8f9a7f512d42fe7ab9	Dinoflagellata	0	0	0	4	0
f2b8ea3ecc75be1e2d17f1437128af19	Dinoflagellata	0	0	0	4	0
27a8b0bf947fcdb944214fd81de30fc0	Dinoflagellata	0	0	0	4	0
01ec223c8a7fab53cd21ac6be3fd6af9	Dinoflagellata	0	0	0	4	0
6a1cbf8e80128e00a54974405eb58104	Dinoflagellata	0	0	0	4	0
67c0e0cfba72f83250f4fede02e6c09b	Stramenopiles_X	0	0	0	4	0
9a0c2e37118ed3188a6c180595984a13	NA	0	0	0	4	0
8ef28f2772250616c83b90bc6c69daf6	Eukaryota_XX	0	0	0	0	4
ca3686cb8c21031f087ab164fdf38942	Dinoflagellata	0	0	0	0	4
3bcf4b6156c1bd8c2c4ffbc9a1580570	Eukaryota_XX	0	0	0	0	4
831f21b89ba084dcb6184306b0e6ee5f	Fungi	0	0	0	0	4
e6e8a4afeceac6b623a6494315811e93	NA	0	0	0	0	4
467db677d42fe4be6cdc9a34231bdc1b	Metazoa	0	0	0	0	4
ecbccbd4e8dcda238594d0f67c0272e1	NA	0	0	0	0	4
82bad4a93311b1971de3a9465bf6899c	NA	0	0	0	0	4
28e4ad41a1916b8ce974193a9f0b4c57	Stramenopiles_X	0	0	0	0	4
8309323a71563727d3b46b98baa51e3c	NA	0	0	0	0	4
8fd739c49d5fc995d869636818b7601c	NA	0	0	0	0	4
b09f4dd1f7ec02aa79586f64fc50d217	Eukaryota_XX	0	0	0	0	4
4ccebfc18647cab7246ac01261f97334	Cryptophyta	0	0	0	0	4
55424a106e8a332e556ac94c588cdfda	Ciliophora	0	0	0	0	4

39aec718830cadae57b90cf3da17b8e6	NA	0	0	0	0	4
1ff67f63ad866ea0de0e382d35b08fe6	Apicomplexa	0	0	0	0	4
b6a85cb18b0eb79cc266660f7301f0bb	NA	0	0	0	0	4
05babc522fa56d21a04159b03f81b7d6	Eukaryota_XX	0	0	0	0	4
c3a2fe1bd139fbbbe995d75bfc35eaa7	NA	0	0	0	0	4
15e65079d9e5c456c34df3a108673e85	NA	0	0	0	0	4
eb95833111125b9c4d413659b31e94ba	NA	0	0	0	0	4
7197066d2018e40e92d32b7207fcc65a	Eukaryota_XX	0	0	0	0	4
5bafaf467ca5c28665bc585663daa333	Ciliophora	0	0	0	0	4
68aa85faa30ccdcd9d3b83185be8aee6	NA	0	0	0	0	4
a040bc8f0d32732995ca34863453860d	NA	0	0	0	0	4
501e14fde8805bb4dff0c89f03140751	NA	0	0	0	0	4
88a9bfb995f6258dc36c9343415bd014	Stramenopiles_X	0	0	0	0	4
01cbb784a21e7b2d5ee2d579bce21d50	Ciliophora	0	0	0	0	4
2f1944d2007cb225193f65acbb506d03	Dinoflagellata	0	0	0	0	4
7f3bdcb774f6dd37e55a68f7921b9d03	NA	0	0	0	0	4
c44bda647f78fb84a63f329c3a956038	Rhodophyta	0	0	0	0	4
73d0f84067ce1245d154e243f5aa4563	Stramenopiles_X	0	0	0	0	4
a3b54168725b23de1c09ec4916a63b38	NA	0	0	0	0	4
a395169e8ee8f55ba88f2af6c36d6c06	NA	0	0	0	0	4
1cd37f8aa06531a2a741d6286cba3ded	Eukaryota_XX	0	0	0	0	4
089840a6ef697498f2dcc46fdf805f44	Eukaryota_XX	0	0	0	0	4
73e70fac93b9b4d80d8f8daba999744e	NA	0	0	0	0	4
8dedd1c6fc473d1f2694160740d3738a	NA	0	0	0	0	4
d15cb91f5caf2c0f8f704c63572fcc76	NA	0	0	0	0	4
97d2a539a8f7a180fe110fb2deb71fcf	Dinoflagellata	0	0	0	0	4
c65561fd8759206e6a79412706a18512	NA	0	0	0	0	4
62059dd15e6f505125fb099d7f10df7b	NA	0	0	0	0	4
3eb24d43a0573f59ae3888746c296a97	NA	0	0	0	0	4
8791d4a90883b8a09e45ea1fcab6d856	Choanoflagellida	0	0	0	0	4
7913fa67fa1ef84dac9e4a52b98794e9	Metazoa	0	0	0	0	4
83c0ac50dec862d2c0d2d251e6197677	Breviatea	0	0	0	0	4
27c0f153dd2d3e60f9b202d78accc568	Eukaryota_XX	0	0	0	0	4
fcf61b5d27eb6b51c1d9e497906cf80b	NA	0	0	0	0	4
a814b57ef9739df7105d49bd57f60807	Eukaryota_XX	0	0	0	0	4
642cb6b5616606f9adeebae4dba328e2	NA	0	0	0	0	4
d710f44e0625a0d45c0bff976780baf0	Fungi	0	0	0	0	4
960436105b7c639099e3c62ead9fc9cc	NA	0	0	0	0	4
79ff2c223e96d7bf9ee3deac071c956c	Ochrophyta	0	0	0	0	4
d6844be53bd1348fa7de402c5a5bc805	Eukaryota_XX	0	0	0	0	4
d61984667b650518c3f725d686b68247	Dinoflagellata	0	0	0	0	4
9c32e7aecb8192527a6feec0e05f954f	NA	0	0	0	0	4
0894e6ea46c1306cc8aee732de7545da	Eukaryota_XX	0	0	0	0	0
0a7a861fc2cb7f89ff46ca608a94ac63	Dinoflagellata	0	0	0	0	0
3cea531b582983cb58342445633d2237	NA	0	0	0	0	0
b6ea1173a6befdcb436aaa3785a1d4ba	NA	0	0	0	0	0

25bd51c427df7e58cc589ce1378a43de	Eukaryota_XX	0	0	0	0	0
4b924d71267e42cca5899224eccac339	Eukaryota_XX	0	0	0	0	0
05af1e7d63d23253bdd77c1aaaa9e12c	Dinoflagellata	0	0	0	0	0
4fe779da0f6bab2d682b954c969b8746	Dinoflagellata	0	0	0	0	0
4a559bd5cd8a928e30cdda9c71c60ef9	Cercozoa	0	0	0	0	0
1e7cc2fb8c4e2d8e1c1df76b08639f7e	Chlorophyta	0	0	0	0	0
77100f94625753bf767b077269361edd	Eukaryota_XX	0	0	0	0	0
577c461ff353d7bc7d1a07444c8d025b	Dinoflagellata	0	0	0	0	0
75d64bd1e9b667ac64515748ff8017bb	Dinoflagellata	0	0	0	0	0
d591b68154dd6f84ccfde4f4bdca7122	NA	0	0	0	0	0
730e23b757103bad8fb3ec38876a904d	Dinoflagellata	0	0	0	0	0
03e7c5956a9832263d62530e52d14dd4	Fungi	0	0	0	0	0
86e62f2df6d3ccfacbba6bc7bbf1ca65	Dinoflagellata	0	0	0	0	0
fe4dba81aaf37503e879ed103d19f4e5	Ochrophyta	0	0	0	0	0
77769c6987a902c373de565280882df3	Chlorophyta	0	0	0	0	0
bef4fe4e93ad444095fcb715b071d762	Chlorophyta	0	0	0	0	0
41fc23a73cf52dd4af4d5a0c868101ea	NA	0	0	0	0	0
f1aa226dc5e292db21588fdcefebbeca	Dinoflagellata	0	0	0	0	0
eb79820a1830f0af6855aaebedb730ac	Dinoflagellata	0	0	0	0	0
7db37aedda9a3fc07eb5923b03a443b6	Katablepharidophyta	0	0	0	0	0
1b6e74f2949d62d83ce1371596619a5b	NA	0	0	0	0	0
e3b63a10da54ac9a16b3593e93b7e076	NA	0	0	0	0	0
0ff6373a69be7b9c229c7cb0c216e98d	Dinoflagellata	0	0	0	0	0
584998b3b648c538b4cce15326b5b1da	Dinoflagellata	0	0	0	0	0
4074b3116b5d3cdbb21e9c5594340472	Dinoflagellata	0	0	0	0	0
0b8beb49069851ed918176f473075978	Metazoa	0	0	0	0	0
e2e7b56a461c697d6eabc302ccb7c0c6	Dinoflagellata	0	0	0	0	0
df941383f2d2d1221d181bb182a33172	Dinoflagellata	0	0	0	0	0
83fd5e9666cd33e16d273a16e004265a	Dinoflagellata	0	0	0	0	0
6f8bfd478218c1d3e00136b736c2ab3b	Dinoflagellata	0	0	0	0	0
5846ccd4535b78e76e86175456cc078b	Dinoflagellata	0	0	0	0	0
f6a8a240ed430fbdea5d1c8aaf9d2bda	Dinoflagellata	0	0	0	0	0
acbb7bfd3cedcbb19740e9430ab71807	NA	0	0	0	0	0
931831fe8b12aed672c6f0265a9060ed	NA	0	0	0	0	0
71b86a524c511ee628267c2368e9588a	NA	0	0	0	0	0
4f7d1c95c3f130c914aad1259c1bdf4b	NA	0	0	0	0	0
6d2901360ee7953f8cd82e518302f6ea	Dinoflagellata	0	0	0	0	0
c160f31c57974b510dfb414abe698213	NA	0	0	0	0	0
3c95b98341c0d2bd4000b2b100fa2fa5	Eukaryota_XX	0	0	0	0	0
759dbd151bc7c5e7f9c748f6f42e4b3f	Dinoflagellata	0	0	0	0	0
0b60cd9f0d4da3b8039ad68c47e850e6	NA	0	0	0	0	0
7d66748cb5ed843a6f5dee6e1ef80592	Dinoflagellata	0	0	0	0	0
92714ea0157166d560bc23158ca30f28	Metazoa	0	0	0	0	0
c65fd42db10f5854e00d96dbe5aed574	Dinoflagellata	0	0	0	0	0
048c35dfea3450327f5aab8a7de25cb2	Metazoa	0	0	0	0	0
7d65e50c9da3896027be20e611b86310	Apicomplexa	0	0	0	0	0

c0e79c3e786aaaec74e6f4faa9399cb2	Dinoflagellata	0	0	0	0	0
e31b16d66ecc0a19e65698b0a00a5ff5	Dinoflagellata	0	0	0	0	0
a90acd12eaddc56318f4496089931fac	NA	0	0	0	0	0
8fd78085d12aa5aff48727248704746d	NA	3	0	0	0	0
3e56a3ee64f8712fdae8e808c7cc7565	Metazoa	3	0	0	0	0
4170554b5f966dfcea8569dbe5b877f8	NA	3	0	0	0	0
0083a4b023fc17db97d0f6945c7fe334	Dinoflagellata	3	0	0	0	0
68d8186b1d0279130c066dc23ab446d0	Eukaryota_XX	3	0	0	0	0
de12dacdcfc6b722a46edc86870598ff	Eukaryota_XX	3	0	0	0	0
862c7bcbe9f8eb843b7c1ffd829d72d1	Eukaryota_XX	3	0	0	0	0
a3ceccd2055cc1618ebfbd55fdd0c22e	Ochrophyta	3	0	0	0	0
13afa35884aca15a41c2cbb2d7a9b477	Eukaryota_XX	3	0	0	0	0
c630cfe6771a2e292aefce8838b3b6e0	Dinoflagellata	3	0	0	0	0
81f8eace8bb144ed2e4693f9f88ddcd3	Eukaryota_XX	3	0	0	0	0
5bb87af0b794bf6dca72137b0daf5dd6	Ochrophyta	3	0	0	0	0
78daee2cd1d51bed7caf62ea2d12a748	Eukaryota_XX	3	0	0	0	0
580b3d66a0c12cf69f3f39716677e483	NA	3	0	0	0	0
c6ce1b189387d36524685e450e8b30a4	Fungi	3	0	0	0	0
f4c809e266591f4fbd3890a906fbbac0	Eukaryota_XX	3	0	0	0	0
a6819148bac9b4ff59962bebdf8f3b43	Dinoflagellata	3	0	0	0	0
3bc30ac538c4109dc7ef725028b26832	NA	3	0	0	0	0
b6eea6eab02da1cb3de0b0c2975266d0	NA	3	0	0	0	0
6e790383f03c98baae0938b6608d3c15	Dinoflagellata	3	0	0	0	0
47c83020aa653670ccdb05e1e1115208	Ochrophyta	3	0	0	0	0
4a8a42bb71ebbb6ae34cd638ba46c3f7	Eukaryota_XX	3	0	0	0	0
11ec1c3376d99e7a54ae03356b393281	NA	3	0	0	0	0
d776aa3fa086e54a7f67b84f26434aa5	Dinoflagellata	3	0	0	0	0
ce0ff38455cd15d9b4e9a25ef719c79c	Dinoflagellata	3	0	0	0	0
866a823387a245c6295a2e07f7d5674b	Ochrophyta	3	0	0	0	0
aff270712483c2a382c5466dc13b812f	Eukaryota_XX	3	0	0	0	0
739bec5cb29a30c78543609de9c8b711	Dinoflagellata	3	0	0	0	0
efa251cebf09ff6aa62f0f29918440ab	Eukaryota_XX	3	0	0	0	0
b0d8880c8a155bf847a6cf6b8d51a3a8	NA	3	0	0	0	0
d6aaf3911453d3bff2045150612a52ba	Ochrophyta	3	0	0	0	0
3c4049ebf25ba510e6b90fc18f3421e9	Eukaryota_XX	3	0	0	0	0
cec5c401be0b32d6ab068da81795498d	Dinoflagellata	3	0	0	0	0
b32cf8224b672f1791d168d619fb9a3a	NA	3	0	0	0	0
2244213b10793f043e683ee7a07abfe3	NA	3	0	0	0	0
54de113981b173b2b95730977a6a6fa0	Ochrophyta	3	0	0	0	0
b08b6aa0b743b38d40da0908c85ac533	Dinoflagellata	3	0	0	0	0
fcdf76e2d3113d9470a92631811f80c7	NA	3	0	0	0	0
dcce06c3640cd508f52fe738bacc25c7	Eukaryota_XX	3	0	0	0	0
02fdbe4bc4247c259f34224fc6594056	Dinoflagellata	3	0	0	0	0
d2ec8d400ab46c1df39f910db5e42cf6	Cercozoa	3	0	0	0	0
ba5dafc8564cdf6311b577507835c9b2	Metazoa	3	0	0	0	0
cb2afbb291fddb59d5203c2712b38d11	NA	3	0	0	0	0

5bcc5793559035a2f5ea841947a8d492	Eukaryota_XX	3	0	0	0	0
0df73bb125bf70530889a5f791f58caa	NA	3	0	0	0	0
00163ecd11909cb11de9ed8977697689	NA	3	0	0	0	0
1a25a2127c82e7e44c3fb2d2cd1d112a	NA	3	0	0	0	0
aa43b9c6a48b127d35cc277058c9e805	Ochrophyta	3	0	0	0	0
fa4dc9173f42a0bc4c8c51672adce824	NA	3	0	0	0	0
3727943c5b6b3f6b86ca8151b7d9a069	Eukaryota_XX	3	0	0	0	0
8d846a8da300f2ba75a1708245f7b04e	NA	3	0	0	0	0
88357875cdc19fcecff55e97e2f30751	Dinoflagellata	3	0	0	0	0
4185abfee42a8c1abf6c94e01e74c89c	Ciliophora	3	0	0	0	0
2bc6ee387995b6feb0582c608ca5f6f5	Eukaryota_XX	3	0	0	0	0
6b9abdc7b953786aade344de22f3b058	NA	3	0	0	0	0
c657292eec7ef4161f9eed952c6e16ae	Eukaryota_XX	3	0	0	0	0
775f17e986123ba680db1f9a1a681976	Metazoa	3	0	0	0	0
b41ac5c511c8cdf66b19e63e9da4c977	Ciliophora	3	0	0	0	0
22e1720bf7c5d82bed58c454d052643d	NA	3	0	0	0	0
052a7f2222baafaed14a11f38760df1d	Dinoflagellata	3	0	0	0	0
72f4f58fed51206dd89a9426e0ce1359	Dinoflagellata	3	0	0	0	0
1c5940016dffd98e97c6d1061fae32f3	Ciliophora	3	0	0	0	0
b8eb23dfaf2d7350ada46dbd508c0b83	Eukaryota_XX	3	0	0	0	0
468c0fb2aecbd356f3dbb3cfcdbca2b9	Ochrophyta	3	0	0	0	0
105c4cea49c7262757f521f346af4c38	Dinoflagellata	3	0	0	0	0
3121ff2451e9f779248b4284dff12db0	NA	3	0	0	0	0
03deb55d1ae7f5f70943fca43e7a7fde	NA	3	0	0	0	0
602f1b9091c0676ec459184c863a6f4f	Dinoflagellata	3	0	0	0	0
9894fe846a88d9117ae275269b08a5ec	Dinoflagellata	3	0	0	0	0
8e81c1c84620ac2898493f59c5673fb1	NA	3	0	0	0	0
be8c4ffd06af31ae3a93e1c2d999f66a	Stramenopiles_X	3	0	0	0	0
b6a64c834fd4f87f5183a9d312fa654e	NA	3	0	0	0	0
2c9b12e83c31466704ceaa8db7b730b5	Eukaryota_XX	3	0	0	0	0
673eab9351c645f22cf18d042566cd2a	Dinoflagellata	3	0	0	0	0
1f6fd4c6a5cba759c389944a6a5716b5	NA	3	0	0	0	0
81b653ee47b06ca661b769d57fd0842d	Eukaryota_XX	3	0	0	0	0
982f60e42ea82f88cc8812ffd2002a25	Ochrophyta	3	0	0	0	0
c9ff84fc9428db493496badfd5521f8e	NA	3	0	0	0	0
256099ece9ed132e9ece85345f1e593c	NA	3	0	0	0	0
564d645f8a10f67fb4bdcd05e218f006	NA	3	0	0	0	0
97c9aad0dd475d13567d2df519b18270	Eukaryota_XX	3	0	0	0	0
da205f7177775ef841c971bedbbddc24	NA	3	0	0	0	0
003dda1a36c83d3c34fc6af0c6b95928	Dinoflagellata	3	0	0	0	0
711a8f1040a83ff5f5e4dc185e63380b	Eukaryota_XX	3	0	0	0	0
cea298703413d6fd1e6cbfb5247a9832	Dinoflagellata	3	0	0	0	0
5e7ef9e4475fd845d7e8307fd610d5fd	Ciliophora	0	3	0	0	0
8c954fddb3fb3cd6e8a393889a9d7b1d	Dinoflagellata	0	3	0	0	0
e726cc955d7f6be8417ecc8ea3dd9800	Eukaryota_XX	0	3	0	0	0
bd5bfc9abb27d782778e84b42340ab87	Ciliophora	0	3	0	0	0

4e959fc195b8fd92fc2fe4445eb40c96	NA	0	3	0	0	0
27a4fdfb99c0661650aef34c4967ddcb	Eukaryota_XX	0	3	0	0	0
c77b1739e04bab4c2157fd239d9f4416	Dinoflagellata	0	3	0	0	0
32a05fdf1588b458b632be02205b7f8b	NA	0	3	0	0	0
6ecb647f72037e6f26e9f2968fda7775	Ochrophyta	0	3	0	0	0
bd3c38dedc8c9bde56b063501f75c2ac	Dinoflagellata	0	3	0	0	0
ed0b8e6c215d8d4a2e136c88ed01ad05	Ochrophyta	0	3	0	0	0
425efb056e231e00c799cc6c8e014066	Eukaryota_XX	0	3	0	0	0
272b73f17f81c755869a8d1171cab260	Dinoflagellata	0	3	0	0	0
39d9b92ec45858083271b46e1cb72012	Eukaryota_XX	0	3	0	0	0
02c94d858f3f98d215347df89c818635	Eukaryota_XX	0	3	0	0	0
22d7caf235f3a1b2b7e4d0bd32827acc	NA	0	3	0	0	0
c6371f68f9c588b5b6d52da973931923	Eukaryota_XX	0	3	0	0	0
7621c15d5eefbaeaed61f907fda91879	Eukaryota_XX	0	3	0	0	0
872f82819a2b8155be3f61eb1bc7a13a	Eukaryota_XX	0	3	0	0	0
26d9c5f22e1f058df5902306a739c400	Dinoflagellata	0	3	0	0	0
72db777116d4b173d31f31c5ff729f80	NA	0	3	0	0	0
df7afb8c6607c92ab5fe070932d77295	NA	0	3	0	0	0
1011dce5ac99985496722997019c0f19	Eukaryota_XX	0	3	0	0	0
d90a38f777155c123fa204977d1fca74	NA	0	3	0	0	0
305dc30236b227a28e0195d5bb6ae264	Eukaryota_XX	0	3	0	0	0
076378e963b80eef34c77a1b06078545	Stramenopiles_X	0	3	0	0	0
adac7443d6beb2c988cb3d0c0e2e236e	Chlorophyta	0	3	0	0	0
8637aa9d497f3ea34084b90c2ea9739d	NA	0	3	0	0	0
a17768c94c508137573275cfb05705b3	Eukaryota_XX	0	3	0	0	0
9fb887386720f9483486c73da0e63a46	Dinoflagellata	0	3	0	0	0
dd9fbc2d09da34d42a10fea0fdc02eda	NA	0	3	0	0	0
4b7ed1d40e1d58726bac8dd1f384f594	NA	0	3	0	0	0
076f27642928d9c86f3ad1cf55f93f49	Eukaryota_XX	0	3	0	0	0
24e7a589a9f8ea603556c536302e5ea0	NA	0	3	0	0	0
e1b7092d80244070723b1abf0391080a	Eukaryota_XX	0	3	0	0	0
0c83c9923f3a9ea6af4086fc7d3b0ea1	Eukaryota_XX	0	3	0	0	0
225064c052d03cfd0fa94d3f90a0141a	Chlorophyta	0	3	0	0	0
8414a870de8e778aad4443c5ad062902	NA	0	3	0	0	0
5f9d20767a2336c560422a0c40fc2454	Eukaryota_XX	0	3	0	0	0
c8150a642f6ee08bc1256336fcc17add	Eukaryota_XX	0	3	0	0	0
75c1d24b9155dcd290d3eeb76a9f435b	NA	0	3	0	0	0
d573c2f77a0a2161c9e944536ed3b39e	Eukaryota_XX	0	3	0	0	0
79a16b62332b1e716585f39f89ee33e8	Dinoflagellata	0	3	0	0	0
068c6e0561e12fed40f57c898b219f36	Dinoflagellata	0	3	0	0	0
97b47318b3eba42bd8edc1eec3fa2461	NA	0	3	0	0	0
f26759b39d2e3cdb0d1ba4e0df5a0f63	Ochrophyta	0	3	0	0	0
c9c28b542a033f9a6b80e730cb95c37a	Eukaryota_XX	0	3	0	0	0
e8475c5d4c8913a339268b625d09090a	NA	0	3	0	0	0
445ce8afdc3b6c25098bb11358f56113	Metazoa	0	3	0	0	0
4a5e655c8a39c92ee5ceeea79cdb5453	NA	0	3	0	0	0

8d9b65df6dfa9ac24d8baa81d9e2264f	Dinoflagellata	0	3	0	0	0
eabcc0c69a28f4878719a9ed6e9a8493	Eukaryota_XX	0	3	0	0	0
2fa9f64bef047e3a817eafc5bc4bf9ed	NA	0	3	0	0	0
78a07b31c271aa425b29e763084b70fa	NA	0	3	0	0	0
70bbf36115d5bf8734cf7419b0cbe167	Eukaryota_XX	0	3	0	0	0
a77353b2b84874fea4ab48bc33add7c1	Ochrophyta	0	3	0	0	0
66cf32264ffa1be2fe87c3b0d51006dd	Eukaryota_XX	0	3	0	0	0
57268609739baddd397c7ec1e4f7b077	Ochrophyta	0	3	0	0	0
bdcb213fd29a5dd91aff6e197d5c8efd	Eukaryota_XX	0	3	0	0	0
f2f5574d5db34cb9a2c3d2369097b2ff	NA	0	3	0	0	0
27e2d34adb2eebfb9d2fc2a353e9e19e	Eukaryota_XX	0	3	0	0	0
1d7494bd7e78f3408cbeeadd0edbc366	Dinoflagellata	0	3	0	0	0
4afa1c6d61137581a027c4039ec445d7	NA	0	3	0	0	0
b25b1909fb5aac621805e9807f7fe226	NA	0	3	0	0	0
e73e2955c8a4f4d8a07c7b5b3ec84fdd	Eukaryota_XX	0	3	0	0	0
8f67f03ccaf29011841198f08e14fcf5	Eukaryota_XX	0	3	0	0	0
8f6e40d702674e3330b94759d2a0ae0c	NA	0	3	0	0	0
ab1be779f8a67ac98a0e47025d7a3561	Cryptophyta	0	3	0	0	0
14928ab2b8c895d76d74a8459d1244e0	NA	0	3	0	0	0
983d269590e21f539ab04ce391a43788	Stramenopiles_X	0	3	0	0	0
215ff2f54626f2f45c6d5e39e6389912	Dinoflagellata	0	3	0	0	0
356bd9a8b5b362c7afd8a57836b37e5a	Telonemia	0	3	0	0	0
858e2c6d8d75074edbecc1269850e555	NA	0	3	0	0	0
6d8dd8ba6c803386184b6ce2795561ca	Stramenopiles_X	0	3	0	0	0
f7d80cb634c7db890117b531beaceb51	Picozoa	0	3	0	0	0
aaf8d2c553cae5b232d85dcea35ef8c3	NA	0	3	0	0	0
05b410d910eaeae650c5766bf0a7b3be	NA	0	3	0	0	0
1e8b841cdeacfd4d33e8301a10939abc	NA	0	0	3	0	0
567b8342564b77fbd22f35160ee28edd	Eukaryota_XX	0	0	3	0	0
312de9faac53d307a6c59c2a69b5de96	Dinoflagellata	0	0	3	0	0
d8b84bb481fce41ba124ee72197748a4	NA	0	0	3	0	0
955741172f0a2769d761a8a4496625ee	Eukaryota_XX	0	0	3	0	0
01ed5b457ae85499c0c3ceb7247c8ab2	Eukaryota_XX	0	0	3	0	0
65cac1343178d04938cecaf87f805ec4	Dinoflagellata	0	0	3	0	0
120171a322d2938b1d9355eafd808091	NA	0	0	3	0	0
6d9c3798c64222ebb9aaaf750b42cb9c	NA	0	0	3	0	0
00b211857d77bb85d32545c852c45e94	NA	0	0	3	0	0
4c8def930f1943273fe28f27a88467f5	NA	0	0	3	0	0
507bf2581ecf3e3b35311bd263c49df4	NA	0	0	3	0	0
964ee816fa0dec3f70a489925e7b32b1	Eukaryota_XX	0	0	3	0	0
a79ce1b1e695c34cec45461395192b34	Eukaryota_XX	0	0	3	0	0
df6350e31b92fa9a29b9302226baa385	Fungi	0	0	3	0	0
f9f68ac9732f928b79bf4f3f141f159b	NA	0	0	3	0	0
4f87449da7dcf624264565d531eb9af4	NA	0	0	3	0	0
73e54db810c852f381d58a93bfef5841	NA	0	0	3	0	0
6cbb693170a2c9c951ff6f7ea34a8ab2	Eukaryota_XX	0	0	3	0	0

538db17f601164336cc33f42a3f8d466	Ochrophyta	0	0	3	0	0
32428b15939acd7af0a4fc23c3d0ff52	NA	0	0	3	0	0
648b917ad136555690afe87054421bef	NA	0	0	3	0	0
e03024ddf4fd56ca27bf3a68fcd7e856	Eukaryota_XX	0	0	3	0	0
30a18a17e4cf39eb698f742c1fa29cc7	Dinoflagellata	0	0	3	0	0
65cd58dcb47823e1c91e38a734816f72	NA	0	0	3	0	0
8b42f2bd0cd4c85b76e0284f311b8e6f	NA	0	0	3	0	0
eccd90ca60f3273885d066dc053b1c4c	Dinoflagellata	0	0	3	0	0
10cda1a878b0ccc879711ccf9f6deba1	NA	0	0	3	0	0
d3b1da6fcd9a67bced3307b2f228e19c	NA	0	0	3	0	0
3dfac98b2e42a957c1d585359edbc597	NA	0	0	3	0	0
63e2a91193e633c74bf25109d9373795	NA	0	0	3	0	0
723de30f8fee7f93b12c3b77442d8872	Eukaryota_XX	0	0	3	0	0
5f478854f27004b56d1e8d2695c2b961	Eukaryota_XX	0	0	3	0	0
a6ffae25f73bb11a426849f62d71cc33	NA	0	0	3	0	0
d2a5440bb393957274f07a309235730b	Ochrophyta	0	0	3	0	0
313ffd9a12eeb4e48a3592aad2ba9382	Metazoa	0	0	3	0	0
4547524d90f405903c9045c43237159c	Choanoflagellida	0	0	3	0	0
aee923bf3fbff96156fa272ceadc3176	Eukaryota_XX	0	0	3	0	0
f582f4104119fc9a368805ceae061077	NA	0	0	3	0	0
5c51468e95af3f0975bcc4a6140054e8	Dinoflagellata	0	0	3	0	0
34e79252eed5fc521524aaae57858396	Eukaryota_XX	0	0	3	0	0
55201616304a02ae69ad38c36027bc42	NA	0	0	3	0	0
177e2e7dae994e5b1c1343837ed45bfd	Dinoflagellata	0	0	3	0	0
7f84cdd8a666efaa0df822c591085347	Eukaryota_XX	0	0	3	0	0
bdb75b69f82c45690122cca79cc1cf1f	NA	0	0	3	0	0
289a56161dba773e393a2327bc806535	NA	0	0	3	0	0
61f9f255fcb7fd5e04d56c513ef3ab25	NA	0	0	3	0	0
5dc7c46b2bfd62f61937f1b48f7b98dd	Dinoflagellata	0	0	3	0	0
2fb1dcd39e322eb83d9519d5b24494fb	Dinoflagellata	0	0	3	0	0
749d0722ab2ec671919c124ddc2e3574	NA	0	0	3	0	0
cf3b51fa95aa3490e059e278ce4fc1a5	Dinoflagellata	0	0	3	0	0
7d315cb17bcfb8b6f02373c48f640811	Eukaryota_XX	0	0	0	3	0
36bb7de1efd88b8678f662d3ba2a1b2a	NA	0	0	0	3	0
e4a27e7b4185189a43792c6e64d9cbe4	NA	0	0	0	3	0
0836b0b0c28b4704062c81cd527edfcf	NA	0	0	0	3	0
2f065a8283c4691dd9623ee8abaf5ada	Dinoflagellata	0	0	0	3	0
a6d54d875b57015798bdbe57114c1274	Dinoflagellata	0	0	0	3	0
ceb9b87dd4904ec5b0189ec821b08db3	Dinoflagellata	0	0	0	3	0
7e52480aea8e817725755eedfa5ed026	Dinoflagellata	0	0	0	3	0
e36846e6583f1923689f51b869254674	Dinoflagellata	0	0	0	3	0
35e0da9e47d2c4382d34e968f5f86881	NA	0	0	0	3	0
aa46f2cfbe842b5b192edd3421537c27	NA	0	0	0	3	0
75430b5e899ce358fa7f4db41c5048fc	NA	0	0	0	3	0
b44fd4a06a5efb08b8cf54e634d71261	Haptophyta	0	0	0	3	0
766774851c818ab8d58a114562ee2f73	Dinoflagellata	0	0	0	3	0

d09d91e8593be3ac12f7fa3334e11f2f	NA	0	0	0	3	0
f4d7bb4e18b350b4cf7d2d310e940d80	Dinoflagellata	0	0	0	3	0
c0196fc3b5b3de79dc7e8b141f930778	Metazoa	0	0	0	3	0
3abb05b8067ce6525f108e414c75eb77	Dinoflagellata	0	0	0	3	0
3e9e0aa52787efd5dbe16bdaeef51086	Chlorophyta	0	0	0	3	0
4f70872f60bc51a776c8f42850ea0687	Ochrophyta	0	0	0	3	0
29625e987a15a9fe0c09c4e2711f2e5c	Dinoflagellata	0	0	0	3	0
baf755b5560fbc7a9b1c3e582ae97dd4	Haptophyta	0	0	0	3	0
7e8f7e36b59f4bf81a52627e149893c8	Fungi	0	0	0	3	0
73a7c9144e17f01a2be87dcd7cdf3362	Dinoflagellata	0	0	0	3	0
c4766b25d764f8b58464e064009837ab	NA	0	0	0	3	0
b5928781311cc0326167a2f23f009bff	Eukaryota_XX	0	0	0	3	0
c952c8d548482a0745fc210c2bc39b55	NA	0	0	0	3	0
a6f481ab72de30544177e78bd28b895a	Dinoflagellata	0	0	0	3	0
f1fba0bec0fccc37f54a5eb3e05cc80b	Ciliophora	0	0	0	3	0
6e79c879a3f2b5a579bde04cb55e5806	NA	0	0	0	3	0
3969b10acee95bdbaa40c8ddea782216	Cercozoa	0	0	0	3	0
b9cabc2ba922fba934aa45bf2341f1a9	NA	0	0	0	3	0
a9a87a31b34961d57b2f1b68a1a2f581	Dinoflagellata	0	0	0	3	0
eba5d3b7d74bfd4f4e9bb302aac9a70d	Cryptophyta	0	0	0	3	0
3bb4bd189e42abab86d1afe7335ee9b1	Ciliophora	0	0	0	3	0
3ae6fa45e1037e67fd4c7e81d79ca1dc	Eukaryota_XX	0	0	0	3	0
5b54c9857a00a415e41846c9780ba47d	Ciliophora	0	0	0	3	0
0444474169921dd1955149d78520cb1f	NA	0	0	0	3	0
ca0680fe5f52af56d0d565ddaf7b9f95	Picozoa	0	0	0	3	0
c9f165ecd2b8dbaf5188ab14e3c14aef	Dinoflagellata	0	0	0	3	0
b4f4f049514be3c3d106dad7a2102c92	NA	0	0	0	3	0
d3fcd58336af15415afcfb0bcdbc0bd3	Eukaryota_XX	0	0	0	3	0
b96bbf969ffa790140a3812e718c64fe	NA	0	0	0	3	0
e13ccbe8f95c7f14a013baa50ab95e4e	Ochrophyta	0	0	0	3	0
418987fedc2eef59db2cef7c1b6ca8b8	Eukaryota_XX	0	0	0	3	0
81a4ef4bcb8039deb83c7fec11e94e51	Dinoflagellata	0	0	0	3	0
7cc16f9bb6822b59901493db937571b8	NA	0	0	0	3	0
edb1c04ffea450dccb7c773c263ffd94	Eukaryota_XX	0	0	0	3	0
48d98b08fd78e0328ae4638361cbe858	Streptophyta	0	0	0	3	0
339d7c0675fe5276bdf138127da1c4f9	Ochrophyta	0	0	0	3	0
54bdfc2e5bca5c0fe67e9692f3855fe0	NA	0	0	0	3	0
492ca014e7300fff2768ba3e1c4d9787	Stramenopiles_X	0	0	0	3	0
d1721249815e61e5d2ee08ecc179c7b6	Ochrophyta	0	0	0	3	0
ce245486202f53a0339303f26b5d0634	Dinoflagellata	0	0	0	3	0
e746dfcd2038a16b36028228b8a57cd6	Dinoflagellata	0	0	0	3	0
6d37137fac05ee56a26c4bcbbd9bfa2d	NA	0	0	0	3	0
db754bf2148dab3d32284b76227a201c	Ochrophyta	0	0	0	3	0
572df0c44b1a130eef1db66cc8699d5c	Dinoflagellata	0	0	0	3	0
c463a37144923ad6752bc8e51ab6976d	Metazoa	0	0	0	3	0
b5796b4a310451c1a4eb035eab943483	Eukaryota_XX	0	0	0	3	0

d6106834b2cc9e5b36436f5642802102	NA	0	0	0	3	0
a94cb26a7d92fdc86a15567a3e9d4521	Eukaryota_XX	0	0	0	3	0
a06215f296e60a22c79dafc0f787700d	Eukaryota_XX	0	0	0	3	0
7f93db4b40fcf55f84fbdbdd315c0f3b	Cercozoa	0	0	0	3	0
f2bdac518d5dab9c84e604d2784f2385	Dinoflagellata	0	0	0	3	0
d6f7d9d6ed0ffb686a8da6a5f6c61d00	Eukaryota_XX	0	0	0	3	0
f7a463fae6d356dc90ed1593503c130d	Chlorophyta	0	0	0	3	0
27d5ece2823297cf0badb6b430e0b0f0	Chlorophyta	0	0	0	3	0
b95576c3295309e5187921edb9ca56d6	Dinoflagellata	0	0	0	3	0
8d7ebec16c44a339f6af46dce33eb479	Cryptophyta	0	0	0	3	0
f9a4e2eff8b1997349dd1c58ffe5e92a	NA	0	0	0	3	0
acf17b55472766572f317d56f90ac24f	Cercozoa	0	0	0	3	0
d7d04b6a0b6801cdbdcfa0040d81549c	Dinoflagellata	0	0	0	3	0
bdb3f2961b6797ccd814446f83879531	Eukaryota_XX	0	0	0	3	0
d952ed2a70fdc9525dc65e8ea3b368ca	NA	0	0	0	3	0
28947514f5a3405cac93d2a8fb048cde	Dinoflagellata	0	0	0	3	0
7bed0806b0c98d8ec348847c2aa18685	Dinoflagellata	0	0	0	3	0
918b405bc5eaf17687513bd1f7a30bdb	Cryptophyta	0	0	0	3	0
37c0ab2c785332872f2838a8d07e4e2b	Dinoflagellata	0	0	0	3	0
e8597fa3274a9547c7d0631da98b1799	Ochrophyta	0	0	0	3	0
a62443dacdf5a339c5d4018b0d35efbc	Cryptophyta	0	0	0	3	0
70c71f438069805feca5b63a35ad8931	NA	0	0	0	3	0
9e26cfed0a2bbcc8224945b45d6b29bd	Metazoa	0	0	0	3	0
7e248c4645683a3711a6cbaba303f7a5	Dinoflagellata	0	0	0	3	0
2ba831b7453f058ebe80da88af16c311	Dinoflagellata	0	0	0	3	0
0669071d78c12e098d1a07bee71949df	Dinoflagellata	0	0	0	3	0
a81277251749940a7aed19b0adddd0f9	Dinoflagellata	0	0	0	3	0
fca9250bce3e136c0d8efc9c3ccda8e0	Dinoflagellata	0	0	0	3	0
8afdd7a8a4e2873e3344f7a0bedfd1ce	Dinoflagellata	0	0	0	3	0
a143246c8c16d8d53361c952cdfab64b	Dinoflagellata	0	0	0	3	0
d993fcb0468414796278e96363a49b6d	Chlorophyta	0	0	0	3	0
3e8d718f564b60ec414a8e78896e7404	Dinoflagellata	0	0	0	3	0
13b2c6ba57978cd686419824886e5f17	Ciliophora	0	0	0	3	0
1b12334dcb80babe0be30707c45b5ac6	Dinoflagellata	0	0	0	3	0
a526dd740db9fe181c3686138e40b00a	Dinoflagellata	0	0	0	3	0
87aff98e44eace8c3eb8b975de0fc14a	NA	0	0	0	0	3
cdf3b8ce0a9c598c5b50f7cc4a19be32	Dinoflagellata	0	0	0	0	3
1e5f8ca015125c9710adf0a685b9750f	Metazoa	0	0	0	0	3
cef600b867fd8f0b65f154adb551419a	Eukaryota_XX	0	0	0	0	3
6060b99eae600b7b107e83018261634f	NA	0	0	0	0	3
a827d23b6cb33cce1cd04765c19d4c62	Fungi	0	0	0	0	3
12577bf08cc14ac30376bd7377de7bcc	NA	0	0	0	0	3
b628bcff05834038b93101961ab23f58	NA	0	0	0	0	3
d63298aa768e5f0bba9575f0abef667e	NA	0	0	0	0	3
a24c1fcac264af91037afae83f6f8d2f	NA	0	0	0	0	3
0b4967d5476b142a90492e683636dd83	NA	0	0	0	0	3

59bd5e838e7adc07e573f0cb12e4b1f6	Eukaryota_XX	0	0	0	0	3
8150cad7dd27a80b05ec112160d264a4	NA	0	0	0	0	3
db1f7108121156bd39220b83c6660a2c	NA	0	0	0	0	3
b7920736b5efb73cba6ce4bfc94d2773	NA	0	0	0	0	3
d198ddb689201c792383c9c7928a0742	NA	0	0	0	0	3
57fb66c5e5260d60a7596ada2e33a41b	NA	0	0	0	0	3
97305ed9c03bfef6c1e12b2392cbc958	NA	0	0	0	0	3
985c2840bd83c44dadaa39fc04d09f54	NA	0	0	0	0	3
30925a740695ae93cb310c59c203523a	Ochrophyta	0	0	0	0	3
b7a9630f892f0eccee1392f4b5f2d064	NA	0	0	0	0	3
7abe11c8968b7720b2563b80fb47045d	NA	0	0	0	0	3
4b4d1d4e86f2f2c159d45d7960f696b1	NA	0	0	0	0	3
cadb9a617c27040ea91755d36d53deb9	Radiolaria	0	0	0	0	3
e60c27908ac2f39aaa02d37923deb8f5	NA	0	0	0	0	3
e303db73345b346ccca2a3118361657e	Ochrophyta	0	0	0	0	3
22ad52030d8f7c3a9f8acfecf14e2428	Ochrophyta	0	0	0	0	3
69d769f70c571ac7d4a5c19fcb072c49	Eukaryota_XX	0	0	0	0	3
57a94e71b8f6348466127593bae37eab	Ochrophyta	0	0	0	0	3
c7be9b7fdfb2ef12786faa37ace79640	Eukaryota_XX	0	0	0	0	3
1f4129568ec604639cb0dd74dc02d49d	Dinoflagellata	0	0	0	0	3
29c89217e956d5b162a182b1930e82e6	Eukaryota_XX	0	0	0	0	3
49d79e0b2dc10b9e42c724686b8f2815	Ochrophyta	0	0	0	0	3
c21b2c28bc3eeca1fd769f18a04dc473	Eukaryota_XX	0	0	0	0	3
28b6a20f3d0fd371055db0876ec48667	Chlorophyta	0	0	0	0	3
3e453406000680b0168376d2d6cf5a48	Metazoa	0	0	0	0	3
d9b3bacc2dc5e664e9a336d18edea10b	Eukaryota_XX	0	0	0	0	3
121ff96eaee677d3ad2f4d8252231731	Fungi	0	0	0	0	3
e538bfd3246171de2b3691cda1a20f61	NA	0	0	0	0	3
768781684fa7ee2e07c94e912f9c5ba1	Eukaryota_XX	0	0	0	0	3
47931cb89a1884e8c3c998ddb7d733eb	NA	0	0	0	0	3
c42f4e02aec184417d67c09a42a38199	Ochrophyta	0	0	0	0	3
03447230da89267db3209cb8cf7ffe87	Eukaryota_XX	0	0	0	0	3
31da5acab3e74ba3051a39a71f54a4e0	NA	0	0	0	0	3
bf1d6ec1a4d353ba2e7e294f8231a560	Eukaryota_XX	0	0	0	0	3
8c471300ef2b34f858e9104c492418cf	Eukaryota_XX	0	0	0	0	3
5c75cecab1a91adf3f79d20d1db21597	Eukaryota_XX	0	0	0	0	3
b52840103053d2edc8f3bcc546970004	Ochrophyta	0	0	0	0	3
c24b59f0b18992a1006734d39505eb5d	Ochrophyta	0	0	0	0	3
943d36821190393f32bd52e6d65d45e3	NA	0	0	0	0	3
14389fec790e300dd431645115afb514	Eukaryota_XX	0	0	0	0	3
12352cf31294dbf872c19402b873b9b2	Ciliophora	0	0	0	0	3
4e31670fc086eb2c694b267c5c44919d	NA	0	0	0	0	3
a2561506038149cb1af9583ff80413e1	Eukaryota_XX	0	0	0	0	3
2320fe180203f7d95140dfed503060c8	Dinoflagellata	0	0	0	0	3
8282d2ca355e815613a6b49facc29e51	Eukaryota_XX	0	0	0	0	3
c6d93e5baeffe2121125dbf3daca3cab	NA	0	0	0	0	3

5e2b44703bcd19907998afcd358c2347	Ochrophyta	0	0	0	0	3
6cffbc109bb4cf4f01b42e1a6b685bda	NA	0	0	0	0	3
55280f65ccd153dc3fc9cb1892d276c0	Dinoflagellata	0	0	0	0	3
ce027ea5a482b9ccb6544ee39dc9f7d9	NA	0	0	0	0	3
30a91c1757281b5dfddd45239d146c5e	Metazoa	0	0	0	0	3
17daf89a47d218e798ab52fa162160de	NA	0	0	0	0	3
cd7afd00b645a9ce1196855c5cab2486	Dinoflagellata	0	0	0	0	3
8880ded46dc60f5d54d263c3aa6bb8d8	Dinoflagellata	0	0	0	0	3
5b1ab8c003d9cf3471238b76ca376fee	Eukaryota_XX	0	0	0	0	3
40739e7432e9d2a6990014ae34f82bb1	NA	0	0	0	0	3
997ee2f371c2f80580475dd0aec1d802	Eukaryota_XX	0	0	0	0	3
e56a67887875b59d37d2206d94a050bb	NA	0	0	0	0	3
86398ed61deeed5c15a8cc188805b771	NA	0	0	0	0	3
4d0bb568382a3a69e0cfc17aadcb8499	Eukaryota_XX	0	0	0	0	3
dbc2f4d51d76c9b7aa92b931e5350013	Fungi	0	0	0	0	3
a9659e20187f82e3c6015ac93498000b	Dinoflagellata	0	0	0	0	3
403a4fa9ebbc2f0dd6016feb3bde5db1	Eukaryota_XX	0	0	0	0	3
b9c224127bae08084b50c19d054c98dc	Eukaryota_XX	0	0	0	0	3
7122a6b8cafb996060685cb3ae8869d9	Chlorophyta	0	0	0	0	3
b7eddc17d4cedb8b0df4c885668dd588	NA	0	0	0	0	3
c863f271d7da8b02ef9a6b6020c532bd	Ochrophyta	0	0	0	0	3
e6f8d1606dc996148606e7c43dc674bf	NA	0	0	0	0	3
7a48c5713a0267ca5c8c2ba28d333d29	NA	0	0	0	0	3
fcf115aa3eefbebf0f8e91ea8c68b068	NA	0	0	0	0	3
83407f249b1e19e959340f62db7e5a4f	Eukaryota_XX	0	0	0	0	3
d77b512c1c91e9d39e7e5a5c19e8ed70	Eukaryota_XX	0	0	0	0	3
a22f2f8bd7ffd6ebf059eddb0799eddc	NA	0	0	0	0	3
28ecea9eee3d8d60557f8fd0d9d74df7	NA	0	0	0	0	3
65c092805def34d160f280ea53865c75	Eukaryota_XX	0	0	0	0	3
282a9c1232b9ce8fe495de6a3d6974dd	Eukaryota_XX	0	0	0	0	3
346bf4c88833dde5120e2a4b484d15cc	Eukaryota_XX	0	0	0	0	3
e33f0e6fe90de625137dc2a8b8e38d5f	Ochrophyta	0	0	0	0	3
fec0410f968e511e6dda2052526f04a8	Eukaryota_XX	0	0	0	0	3
4d8f7bec33038bf97f9bbf57eaa55d7a	Eukaryota_XX	0	0	0	0	3
968d4b23ea5098a9f4a95d88f37e9e5f	NA	0	0	0	0	3
be4d12ad46e627d62be197de2a14b6f1	NA	0	0	0	0	3
86473c24b4330013835f3af7054bfa55	NA	0	0	0	0	3
a9edea7788f22518958deef6e4f76a3c	Eukaryota_XX	0	0	0	0	3
0ffc9cc6e7360aa6df9730b068c27917	Ochrophyta	0	0	0	0	3
8972862acd1fdc6ad37cd411143c1bc6	Eukaryota_XX	0	0	0	0	3
3ec9d244f4990b7811ba37ab0b8688df	Eukaryota_XX	0	0	0	0	3
6151744c8d391f5225af3a3024a812cb	NA	0	0	0	0	3
670de1e3be4a6bdb108a8111193b2012	NA	0	0	0	0	3
c9d87629e23a14b3db3c1c19ce656eff	Dinoflagellata	0	0	0	0	3
d94b480927e4bf6dc16cf76d991db683	Eukaryota_XX	0	0	0	0	3
876b52f6886f29edefd6f8c459105153	Ochrophyta	0	0	0	0	3

28f21c7f2b521d81054598860bd87a57	Ciliophora	0	0	0	0	3
86c956752f7429ad8f4b1faf34d6b39c	Eukaryota_XX	0	0	0	0	3
99fa3f024a8e188c076c832c34c2406a	Stramenopiles_X	0	0	0	0	3
e0b5f76183ec6ffe1ddb0654fc0eb409	NA	0	0	0	0	3
8a7f657bbe8b8260e319136ff3e0f1d7	Eukaryota_XX	0	0	0	0	3
07ea7c4fd0d95854c8aee7b93a1bd14b	Breviatea	0	0	0	0	3
1f989c979fe2644223c9bf705e4e8de3	Eukaryota_XX	0	0	0	0	3
2bca82c8f9f2958f148ca3e6da0a4a9b	NA	0	0	0	0	3
463835f78c0c7a8ea364067e3dcc54f9	Ciliophora	0	0	0	0	3
8b8bca8ce8761d13bfbbdb75174ba1c0	NA	0	0	0	0	3
314de11347f5e0c932ed112572612896	Eukaryota_XX	0	0	0	0	3
dba89aa1e76b29db1d06fa20195bbec5	Eukaryota_XX	0	0	0	0	3
c9ba3060083a94241f1abdc841ce2186	NA	0	0	0	0	3
7891d55934223d683ddb0f516875b9b6	Eukaryota_XX	0	0	0	0	3
8f07ad7e8ff6b9ff99bd0c534a2b58a8	Eukaryota_XX	0	0	0	0	3
bc42e2f1646a2bd77e5e44dd85514ec0	Ochrophyta	0	0	0	0	3
af651ad0c6c53c40eaa122ebd062d281	NA	0	0	0	0	3
8f066cb45aadde3f1aa7b08e21efe6e1	Dinoflagellata	0	0	0	0	3
1800eebfc72b3d31be7a211d92459ef2	Eukaryota_XX	0	0	0	0	3
2ad6bf4c2dda1c94c276d53a213ae4e0	Eukaryota_XX	0	0	0	0	3
4f6445ef42291a5e9d4722ba6674da29	Stramenopiles_X	0	0	0	0	3
90a60c490931431813a77908b0cfcde8	NA	0	0	0	0	3
0b08d20fe70449e0cb287580db524912	Eukaryota_XX	0	0	0	0	3
79676d067ebad9229318cc42305a946c	Eukaryota_XX	0	0	0	0	3
1e03b327720d0e75f9f1bb45dd73ddc4	NA	0	0	0	0	3
a615c2ba1638ee8a758cec024960d5dd	NA	0	0	0	0	3
41db49331f8a12c19c85650923cf2400	Eukaryota_XX	0	0	0	0	3
3da1975adb00367793a831c9253d59bb	Eukaryota_XX	0	0	0	0	3
b62ce07949747dcf0165f5f147967659	Eukaryota_XX	0	0	0	0	3
5d3c043891083b470c7bcbaa9843e51b	Eukaryota_XX	0	0	0	0	3
e6467cfb7849cf1d741149a061e11221	NA	0	0	0	0	3
4a200c8d9cc8a920cee488a9e3bc9431	Apusomonadidae	0	0	0	0	3
2e1a1a9ae2a0d42f8c261b7b9c80f9cd	Ochrophyta	0	0	0	0	3
d10aa56fcd8509cf3593227ed775df67	Eukaryota_XX	0	0	0	0	3
731ae269668353301e85ebf1ae936001	Eukaryota_XX	0	0	0	0	3
25a57894a8281d6c532568f9be3c1397	Eukaryota_XX	0	0	0	0	3
fade1c52db952a7b1fb27eaf742f5a6a	NA	0	0	0	0	3
ce6ada2e69b1400793a1969edab84fe1	NA	0	0	0	0	3
b0ab8049c97f9e5c65ddd39de3eafbe2	NA	0	0	0	0	3
f1f3dd7175ac9bd4b87e467ef4e0e1f1	Dinoflagellata	0	0	0	0	3
443818c9299f5e1c4bf1189f4632e812	Eukaryota_XX	0	0	0	0	3
bf7f65e54f1083db692db5628b4e0c17	Fungi	0	0	0	0	3
f41c371cbde73d676ce14e26d7e48cef	Eukaryota_XX	0	0	0	0	3
a028e5b77931915d8742cb90b7cb86fb	Eukaryota_XX	0	0	0	0	3
d4e5e5fdd6323bd827bd12210a645248	NA	0	0	0	0	3
631b9621675e0d0457a1d1e600275009	Eukaryota_XX	0	0	0	0	3

8c5a8463e63fa2dd02b54b3f52e569b8	NA	0	0	0	0	3
efffb565df1e8d599c0cdae5c21f2fe2	Perkinsea	0	0	0	0	3
da4f66e0146eb19b4da88abf3a93da30	Eukaryota_XX	0	0	0	0	3
6e34c3fa272ecf7bc47ef39f822cb748	Dinoflagellata	0	0	0	0	3
06729fef5d84cda6ee45e9b2e3a4a5d3	Dinoflagellata	0	0	0	0	3
57869007e25c6f1d4aaeeeb8fec9d354	Metazoa	0	0	0	0	3
1b1ba8e9f48e61f1c22f7030f26f5c9f	Eukaryota_XX	0	0	0	0	0
cc6eaaa6873d1c39284671eaa944fc47	Metazoa	0	0	0	0	0
857a6d56e2d1097b13c41bec53664669	Dinoflagellata	0	0	0	0	0
03c5d6f1150ada1ec3f5e6218edfb391	Dinoflagellata	0	0	0	0	0
b328dec94a8ef756b578e3c42ed25eff	NA	0	0	0	0	0
7f0361f26a758a9fed1c1d88bff1e0f2	Stramenopiles_X	0	0	0	0	0
7dbf7f0e6af8118f2f92731bf9a42ec4	NA	0	0	0	0	0
d044f3929534d994ce41f1a99168a0e5	NA	0	0	0	0	0
32195972dc2b6edf093b2e6ab394c887	NA	0	0	0	0	0
88cc48d2c55dba983354a03a780bd26c	Eukaryota_XX	0	0	0	0	0
f843105568de140ca255602a29d6d9cf	NA	0	0	0	0	0
a0590ef72ad2f943081e7a1c3191b734	NA	0	0	0	0	0
85cb34b2cfefb4a41bee0b4c24b48495	NA	0	0	0	0	0
d8a9b9c119b80c46c398d8de9a8830f8	NA	0	0	0	0	0
addf226b87140c4114e1c160286ea2f4	NA	0	0	0	0	0
f2c0f589a335db886f91f5f9a32de2e7	Eukaryota_XX	0	0	0	0	0
d8ab3afac97ea326201ce48768606cbe	Katablepharidophyta	0	0	0	0	0
61ecf3a4b86a354dd305f5eec3b9fbf2	Stramenopiles_X	0	0	0	0	0
fa8ddbe4400771783152f7a5412b20bb	Dinoflagellata	0	0	0	0	0
6bdff05df8158a13c2cd33f61bf2305e	Eukaryota_XX	0	0	0	0	0
6edc1bba69e6a7266f5ac137e2454beb	Dinoflagellata	0	0	0	0	0
0ef2f29f0ce5aa333e8a46c0b5fe9cf6	Haptophyta	0	0	0	0	0
9f45a43049931df3dc1f2f989b4402e5	NA	0	0	0	0	0
3908b96407da210e9ba83166e7d58270	Dinoflagellata	0	0	0	0	0
64eeb47f8c0c1c51b9c66aa61b0bfcd7	Ciliophora	0	0	0	0	0
51c72c33f149cc869691b55c65093166	Dinoflagellata	0	0	0	0	0
88a2f00dd320705ecfc3d8dc202139c9	NA	0	0	0	0	0
d08e89a37fd4914888daee58f4688fbc	Eukaryota_XX	0	0	0	0	0
c99cae2c7665cc0b0f03327f7f495c2d	NA	0	0	0	0	0
e82e14017f00dea0ce5a1ac7c1f33390	Ochrophyta	0	0	0	0	0
ce330aded200467f221a74fa6b5a6787	Choanoflagellida	0	0	0	0	0
4901fbbfecbe2ae44b0336a7294ae9d3	NA	0	0	0	0	0
7d4c467ba0b72fb36e50cdd15bd2a0ed	NA	0	0	0	0	0
e39b054eb8309e4d7e3eca070fe2d384	Katablepharidophyta	0	0	0	0	0
96a06ed7cf73ae352d80c471a341a0f6	Dinoflagellata	0	0	0	0	0
22aa76680191fdb8028533e92b16f1d9	Dinoflagellata	0	0	0	0	0
5cc575999ccc5ec0f9acb484d5f9429f	Ochrophyta	0	0	0	0	0
1956b2077cc1d842a61b948245331663	NA	0	0	0	0	0
a64118f487980b4b367b7f7be5ff2493	Dinoflagellata	0	0	0	0	0
656add4d88a3810302b4de466fe72a05	NA	0	0	0	0	0

20da1235d03c27693e496ceb3e72b9fa	Dinoflagellata	0	0	0	0	0
0c806ce9a6ae87aebc92853a6fdfd021	NA	0	0	0	0	0
2d81ce3eb4358024be1ab44867ebd028	NA	0	0	0	0	0
88dbf0a9488f1f478ae9b4c9611d79d0	NA	0	0	0	0	0
5b2283117582bd6edf6fb7a838e66146	NA	0	0	0	0	0
f5e5b11fcaa86c856c35338ae0ba9ff5	NA	0	0	0	0	0
78ca81cccfe2349c732a61b87d77e8e6	Ochrophyta	0	0	0	0	0
f49c8367739787a683d7eb1f37eb48e2	NA	0	0	0	0	0
6d29b5343b2424a78a1d543999fc4b73	Chlorophyta	0	0	0	0	0
1be5e5061bdf5c49fc1c546949f74593	NA	0	0	0	0	0
60085cd38c486716773f48050ecd61b3	NA	0	0	0	0	0
fb75ec446af2446ef88bebd7b6e5216f	Dinoflagellata	0	0	0	0	0
f6da7a1f208bbc328bae0c6d8eccd3f9	Eukaryota_XX	0	0	0	0	0
2cb8c729b41773c620924c40e2da9afe	Eukaryota_XX	0	0	0	0	0
7df4cd29f5c2d2b66ff8e6e6152973a0	NA	0	0	0	0	0
c18efc1d34ea3a6a9e47560ed7a1ae27	Dinoflagellata	0	0	0	0	0
446569665bca166af758d5ec4fa28ccc	Foraminifera	0	0	0	0	0
895cb00142ff772611f694863d7084e9	Dinoflagellata	0	0	0	0	0
3de0abbf8c90a141652a1d8bb99b8a7f	Eukaryota_XX	0	0	0	0	0
0353409a8da2fd3f8958ea77026f547b	Eukaryota_XX	0	0	0	0	0
c8f3d45e4395c49000d0ab2459445ad4	Chlorophyta	0	0	0	0	0
8963517d8f23f345893d9e791d0ac114	Dinoflagellata	0	0	0	0	0
c49cf1cc914296b2c1c861219d55153f	NA	0	0	0	0	0
53a9b5fd77b02830864a5b08ccc02933	NA	0	0	0	0	0
d7d83ae99bc751c3130577fe7fce21ca	Stramenopiles_X	0	0	0	0	0
7a5b057dd5bf52073a7b081ad0594c27	Eukaryota_XX	0	0	0	0	0
75b1b2d22bae3cdd32fcd2abec966f0e	Dinoflagellata	0	0	0	0	0
87ba4c6f2107a29139a01758f47733aa	Telonemia	0	0	0	0	0
40597a832c0bc28fbb8cc2648eee5b1f	Chlorophyta	0	0	0	0	0
d2a3fba8416ad0d615d38dd2f8a4128a	NA	0	0	0	0	0
90d38adfd6cddfe1fbc20ab83358a29e	Ochrophyta	0	0	0	0	0
41bd322701d725b39502e4d36856420a	NA	0	0	0	0	0
a87924de2c2fd29ed25a2578c3769242	NA	0	0	0	0	0
a486f81b560c2ea7546349cf4a2c3b9a	NA	0	0	0	0	0
0936a646af192342339b3137c82c1ae9	Cryptophyta	0	0	0	0	0
dcf9a1e7dad2704832d8232fb4868402	Picozoa	0	0	0	0	0
1b6636070d64708d73574914c7caea39	Picozoa	0	0	0	0	0
73f3392f09d86fd857130f12acc9f4ca	Dinoflagellata	0	0	0	0	0
b2b238e240865d7ff6617780cce603de	Dinoflagellata	0	0	0	0	0
2bb37090b1b359205b0fa59855e499d2	NA	0	0	0	0	0
bb64a2f5179c2de69dc15694831fb208	NA	0	0	0	0	0
87eeeae7c931f990bfbb7c8415c932eb	NA	0	0	0	0	0
539e4b5eaf91293ea840e447eb7689f2	NA	0	0	0	0	0
7f28f7f336d1e8a0a62ea9217aa6c9b6	Dinoflagellata	0	0	0	0	0
a066d7b3491d9098e2a45a9c3d1091ff	Cercozoa	0	0	0	0	0
3622c33f68b230b6fb2da732f3a64152	Eukaryota_XX	0	0	0	0	0

9a3ba1d02f0f91b95c2ed51263d28790	Dinoflagellata	0	0	0	0	0
9099ea400a9c0f9568729e6c7845406b	Ochrophyta	0	0	0	0	0
cc8b75e77728973fd8977ecfcd0a257d	Chlorophyta	0	0	0	0	0
a6846b919f561de0b9c09545aac65d42	Eukaryota_XX	2	0	0	0	0
e3bbefc3448b3ee0226c6ec1e8f17d03	Dinoflagellata	2	0	0	0	0
e2b5d7c9833f6d84a0e383d75b54510d	Dinoflagellata	2	0	0	0	0
61f861c8ae67327710e90125b4f83f74	NA	2	0	0	0	0
3d508d4f6d4fc349a07afdec69c3ea78	Dinoflagellata	2	0	0	0	0
86c8b0929fc8355f76fefb29437b57ab	Dinoflagellata	2	0	0	0	0
34f11f331e9d138191b16ab0827427fa	Eukaryota_XX	2	0	0	0	0
72801864d4c468fa63ee40ae7848aa6f	NA	2	0	0	0	0
bb3ccb064caef295d8e8de7a9c467256	NA	2	0	0	0	0
e6bdbf05b1ee0046431b456011f2f336	Dinoflagellata	2	0	0	0	0
c0a807f683c4c7a4c387c8100a7c88fb	Eukaryota_XX	2	0	0	0	0
3f1978e917bcb8d7c3afec285eff3e55	NA	2	0	0	0	0
804306a1257ea7e3b383dc4b93464583	NA	2	0	0	0	0
f0a6760febe25b32f60e8cbbdb0a5cce	Dinoflagellata	2	0	0	0	0
22b4044147c8401cb1b71505573d8bec	Eukaryota_XX	2	0	0	0	0
f150cfb12e82683220060cda0342a007	NA	2	0	0	0	0
64d547197fca265fc86b618821b78285	Ochrophyta	2	0	0	0	0
e64937d5cb4c0cc2c3054b58b776b7a6	Eukaryota_XX	2	0	0	0	0
a92d3db464be771a3accad7cf0127933	Eukaryota_XX	2	0	0	0	0
69db2a5681ecfd64a51bb8bc1ea8e0e4	NA	2	0	0	0	0
1dec8b31b4df8141c91d6bed2253072b	NA	2	0	0	0	0
ee5c569730703d3e5a21c9612f01b465	NA	2	0	0	0	0
c50339ac82349668d0630f27f2a64021	NA	2	0	0	0	0
51cfec5b156f891934bb278755c79280	Dinoflagellata	2	0	0	0	0
2a96585b297cbe609548980083e402ab	Fungi	2	0	0	0	0
e17c7d8da0c50712e53f9216eadd3fba	NA	2	0	0	0	0
37b5feaf483bcefb8ee219c52ab3e643	Eukaryota_XX	2	0	0	0	0
7e02507e71f470975bd1917829b9fd0f	Ochrophyta	2	0	0	0	0
2e9da12774579ab66e3164328d80474c	NA	2	0	0	0	0
ba973580e4c0797e0f7181495118ae39	NA	2	0	0	0	0
31105861c329a005f224ad625f14ed1c	Eukaryota_XX	2	0	0	0	0
d6a0a44d594c13a61437ed298cf01294	NA	2	0	0	0	0
7a5049df7256fab741dd3513b7e6aa6e	NA	2	0	0	0	0
b1c394610b1cf6ed83ee420c18a3864f	Dinoflagellata	2	0	0	0	0
456e578f0856b4f0574bd052dd0c8dd1	NA	2	0	0	0	0
5a675f383571557776f87f6d87a05efb	Eukaryota_XX	2	0	0	0	0
33f89fb2a28e461b57c8c747a3218dff	NA	2	0	0	0	0
3d3ca6e0ca05ce8ddb59c11419f17a74	Ochrophyta	2	0	0	0	0
dc79dae90c349f78c58a3e0c4f4e96c3	NA	2	0	0	0	0
ce9f66485f366910225f7169b3967b55	NA	2	0	0	0	0
c5aaea7b0f02e7acc6ad81c7f9d87762	Eukaryota_XX	2	0	0	0	0
0aaf176f4afa29409948cbcc090f1441	Eukaryota_XX	2	0	0	0	0
69ed480e7beace6c73f8ecb4646a7cdb	NA	2	0	0	0	0

22f02b59908ab9f525cee3a8857fda5e	NA	2	0	0	0	0
5c77fca7b572031d070876bf06924e45	NA	2	0	0	0	0
b762e5e029dcbc1a27703d440e94dfa8	NA	2	0	0	0	0
1c7e2488bea59353402edaaffd38b573	NA	2	0	0	0	0
5f04ffcf3f2aed7cda40f636ffdbf955	NA	2	0	0	0	0
8616178170e721d71075b4c403efb5ac	Eukaryota_XX	2	0	0	0	0
6e3d262db0abd89aa2e8742b73b930e4	Eukaryota_XX	2	0	0	0	0
36278c5c8e54b114e7122f1a623b0a63	NA	2	0	0	0	0
a255ed0f1b998abedf11afe1e3477009	Eukaryota_XX	2	0	0	0	0
deb325b73034cab2bb689cbcc4abcd1d	Dinoflagellata	2	0	0	0	0
dbcc41c6f56b0699fa9f9b89e2d6364e	Eukaryota_XX	2	0	0	0	0
57030f0c0f9a297c7daca1ec3a518cc1	Ochrophyta	2	0	0	0	0
8acae5f51c0de1fdc1e5bb63029c8ead	NA	2	0	0	0	0
062be63e2cabd797b159d8dc8fdb46a7	Eukaryota_XX	2	0	0	0	0
2c23a49705480ee1d85cfcf7642d2e9a	Eukaryota_XX	2	0	0	0	0
a4c1acb852cfe1e86b98a23f2cdc4572	NA	2	0	0	0	0
c2567ab8e1789bbbce1167d5bca545b3	NA	2	0	0	0	0
e24fe0cd56ea9a71be689d513bd12b6e	Eukaryota_XX	2	0	0	0	0
ae0a9e92848cdecbb95a3aedcb08834e	Ochrophyta	2	0	0	0	0
30310c1f2f11725bffd72a7f145e8b35	Eukaryota_XX	2	0	0	0	0
d976befe02f855d88df524bf4900a517	NA	2	0	0	0	0
b2d28acb2451c1193251d6b0ce8600b6	Lobosa	2	0	0	0	0
0cb6835d52bb309cf8e7194867be876c	NA	2	0	0	0	0
f62465fa11c86604847227bf4da801e1	Dinoflagellata	2	0	0	0	0
d41ee6e416ed3200fbf6494e6ac6002d	Ochrophyta	2	0	0	0	0
be0fa04db8d820379d829d15c54f95b4	NA	2	0	0	0	0
5d5fae4f59589f5e1aa96f1202343c2c	Eukaryota_XX	2	0	0	0	0
9fa37eade73143521c1dcef0b6b290cd	Eukaryota_XX	2	0	0	0	0
691e8ea081674261aa3028c8fc910f98	NA	2	0	0	0	0
a906b7c604dc6edb9ce5d50b073b52e6	NA	2	0	0	0	0
4dacd2ac39455e7670e688f9fac4b8e0	NA	2	0	0	0	0
8ae489bb9cd545e742c2c72d5ee137f3	Dinoflagellata	2	0	0	0	0
ecee8db49bcd615a06be10cf8a04a0d5	Dinoflagellata	2	0	0	0	0
d812a0d099238e934afbeca05c49f1e5	NA	2	0	0	0	0
5d55928254dadce0b9fe71324eadfa61	Eukaryota_XX	2	0	0	0	0
b566f1d3cd1c8a9b03096c754b6559ba	NA	2	0	0	0	0
04e4a07e2e36295cae77c7a9cb8e71b6	Ochrophyta	2	0	0	0	0
6e7520992c07894e8a0d493cb80f2d26	Metazoa	2	0	0	0	0
76b4e4f5445f2517dd7b72efa9147502	Eukaryota_XX	2	0	0	0	0
303d0cf083d02d1c67beedb291f32014	NA	2	0	0	0	0
4f8b08fea2cf15d9e21d1bc022adcb0d	NA	2	0	0	0	0
983074f2fa6cfeb4a193bf195f70f6ce	NA	2	0	0	0	0
b73b5380f4cb8ab1ee7b0443d26459a6	Eukaryota_XX	2	0	0	0	0
27fd091fb45a13aec0e354692f481cda	NA	2	0	0	0	0
6fc2446d8a9c870eae59f96b1e919cdc	Eukaryota_XX	0	2	0	0	0
b0bbbcc70266724cc701c8e1c56faefd	NA	0	2	0	0	0

d8da0dcf23063ddd5793785f5b860a4e	Eukaryota_XX	0	2	0	0	0
6cb04567feb4178e8f35c9446ab40e82	Metazoa	0	2	0	0	0
669924b5b0aca71fb285a97bba935768	Eukaryota_XX	0	2	0	0	0
6b2371343138f1b4b1b26e785722540a	NA	0	2	0	0	0
ec6e73d6c2f2c5e986d669b60b5fdea4	Fungi	0	2	0	0	0
67d545c39dfc4253d174a24b141c76c5	NA	0	2	0	0	0
ff4718a5ad09b48dd476f0ff7a922de4	Ochrophyta	0	2	0	0	0
c3ff48300e2ed525899ec342831570e2	Eukaryota_XX	0	2	0	0	0
23deeff01533f7d132450ace78161403	NA	0	2	0	0	0
519906ff7a873ea457fb95a745c0ec67	NA	0	2	0	0	0
0fa3dc5c8601c8ea89044b17d159d37b	NA	0	2	0	0	0
267ecac097ba0e47d75ebbb1947de4f4	Ochrophyta	0	2	0	0	0
0b622169ffd7d207455f80cb02ef3b9d	Eukaryota_XX	0	2	0	0	0
f0d8d51cd022131ed4ccce11c5049028	Eukaryota_XX	0	2	0	0	0
32e0f26282070e59e2e0a2baa9246fe2	NA	0	2	0	0	0
52ac27abfa638028387d7a10d902d9bb	NA	0	2	0	0	0
20a50407cd03cdd1ccee75a0cdb31787	Dinoflagellata	0	2	0	0	0
04bf5a4ded80e32bcbbc9993c34d9235	NA	0	2	0	0	0
07db02804c82628dfa21d0d9367d8511	NA	0	2	0	0	0
105ee68b1c2df1c05e748dcdd6ee6f81	Ochrophyta	0	2	0	0	0
4557093ce79db54b0a487b79b7fdd685	Dinoflagellata	0	2	0	0	0
37725fe2ad77d3414be9f79f1882771b	Eukaryota_XX	0	2	0	0	0
00e5c1eb9fd8eef817540de281f6df33	NA	0	2	0	0	0
c17e680e225e282fd980bbd47ca14b37	NA	0	2	0	0	0
2bb3f766ebb314a6088406aebf935aed	Eukaryota_XX	0	2	0	0	0
043fe30fcad3871a4002b147cfc4ddaf	Chlorophyta	0	2	0	0	0
340ce9777a9b5552fe8bf9e0d5a6a0ad	Eukaryota_XX	0	2	0	0	0
31715b3e80479f93b3ca998981d28a48	Eukaryota_XX	0	2	0	0	0
ad4e8b51582659c81dcdc82989b63558	Ochrophyta	0	2	0	0	0
2bdda4feba2a304829c4508f3bc89397	Eukaryota_XX	0	2	0	0	0
40b4909c2c033f6a9a355f11ffcceeb1	Cryptophyta	0	2	0	0	0
62371343061ef7331f7bbcdf07ab86be	Ochrophyta	0	2	0	0	0
9be6b08f4e9393ca6a67d552311d48c4	Ochrophyta	0	2	0	0	0
60447d3a29d12ba9250e64ea70e918dd	Ochrophyta	0	2	0	0	0
c5e88d85487407f0c5c4466419c1dce7	Ochrophyta	0	2	0	0	0
f975061d1ac99162d4e72a8f5bc17e79	Eukaryota_XX	0	2	0	0	0
464c8019ad69a14029173cdfdab55912	Dinoflagellata	0	2	0	0	0
9c3bcdede6cf8d55bfd850570afc92a3	NA	0	2	0	0	0
4d1a23cc2421479ef01812e3cff296d6	NA	0	2	0	0	0
12937247f55a4327e5e471526fe7c947	NA	0	2	0	0	0
e4dc65139884f01aa9c451240a7d2d89	NA	0	2	0	0	0
b34f4a8e4b5121413d82a95b41cb823b	NA	0	2	0	0	0
0327bd7a378788bc7399ec2f28645422	NA	0	2	0	0	0
2ebb10f55bf8dca7f5395191328f857d	Eukaryota_XX	0	2	0	0	0
766c964b6d8ac8f9d3a7170c553287ab	NA	0	2	0	0	0
e803b6f075e06ab737ea9d3e8bf0d776	NA	0	2	0	0	0

48236dd2ced1f48c8e8cd6a1e4fbe6b1	Eukaryota_XX	0	2	0	0	0
79f932ef727c79a3beb9f18fa361daf6	Cryptophyta	0	2	0	0	0
6492baf8498813562c196c17a2f6711c	NA	0	2	0	0	0
5dac10da92c074774aff9c64ebd23dde	NA	0	2	0	0	0
600e1a58f5e37200582001c0e5fa39db	NA	0	2	0	0	0
e120e751a156bbb168e311d724056a99	NA	0	2	0	0	0
dd67ac315485bbab97111900f78404d3	NA	0	2	0	0	0
6eaeaf158516ef79a6b97ff30471882a	NA	0	2	0	0	0
b03088389a6a8266615419054d8907a5	Picozoa	0	2	0	0	0
14b8f016f7b52af4ab273d8428d778ec	NA	0	2	0	0	0
4600e8cc48c4a5755b92fec8f73f1c0d	Stramenopiles_X	0	2	0	0	0
32b2f4a0f9cd02b1d406c6ae3050325f	NA	0	2	0	0	0
89f84f1b3e331fe32d0cf0bec49d791c	NA	0	2	0	0	0
103fd51d2164a638448a7a2ac9670ba5	Ciliophora	0	2	0	0	0
0db0cc7f83d054a189167de854b1298c	Cryptophyta	0	2	0	0	0
ccc167c67f90905322cf2f554b32086d	NA	0	2	0	0	0
49bd4f9020e9d198614334b618ee9327	NA	0	2	0	0	0
55d801cdd9198a478dc752067e3789de	NA	0	2	0	0	0
50786ceb2f6bd066c8b00551d400ced5	Dinoflagellata	0	2	0	0	0
bde6c833e395598e8aa06e0bc4a74b71	NA	0	2	0	0	0
35714a5d0d37116b77d1a8d7409469df	NA	0	2	0	0	0
0d78dad8d7c0dbb957adffe538e401fd	NA	0	2	0	0	0
bc461a319f87f8e9f87fca34c108042a	Eukaryota_XX	0	2	0	0	0
7f2822deebd322f10de8c1396d2eecfb	Picozoa	0	2	0	0	0
a48c49869ae00274fc3a2b7e19574c27	Metazoa	0	2	0	0	0
fcf651ede18fc50f5f6d86ca37fdaf36	NA	0	2	0	0	0
84f904f4c57a4529807b57859a88bc79	NA	0	2	0	0	0
1b067808173e5196c15fc42554397422	Ochrophyta	0	2	0	0	0
799738023329676f37c11cfc36af0b82	NA	0	2	0	0	0
1ec857d24cbc31e2b10ebf938eb97997	Eukaryota_XX	0	2	0	0	0
8bc5d2cc1a5301380013f66e3ab0c61a	NA	0	2	0	0	0
9e71312e1a03e3e4953a1254919fdeaf	Eukaryota_XX	0	2	0	0	0
dc4fb807f75ec159c2d2d9c9b5b09466	NA	0	2	0	0	0
8741c8dc588fd8e960623fcde31e803a	Ochrophyta	0	2	0	0	0
7c226dd91b9ce8be537191b7e23037b0	Ochrophyta	0	2	0	0	0
6d73a79bdafc17e9681576d2f6332849	Eukaryota_XX	0	2	0	0	0
9caa5762d0496be85cb5d1d3ca416040	Eukaryota_XX	0	2	0	0	0
2adae95b27178a44215a2fd1180f2f6e	Ochrophyta	0	2	0	0	0
4d31cfac5a4beb3537a0d8409ec3a80d	Cercozoa	0	2	0	0	0
fa08d40355680ff1b148637c74efe5d6	Eukaryota_XX	0	2	0	0	0
9c253fde353666b798a5861a433fda32	Dinoflagellata	0	2	0	0	0
b25c7a79382ce1d12996320f364cc018	Ciliophora	0	2	0	0	0
76a4b33eed6f369569beef3b90d1bc8d	Stramenopiles_X	0	2	0	0	0
89eec486a4f8ef394f6bcd8c3a8b2efd	Eukaryota_XX	0	2	0	0	0
4384cb8cac910dfd8a3789ba6e540f65	NA	0	2	0	0	0
da8acc8b77b765424afde20672e99c16	NA	0	2	0	0	0

1f2cd9eacaf8ee02a8dba8412ef166d7	NA	0	2	0	0	0
879a28be9531fba839d418b3108c0028	Eukaryota_XX	0	2	0	0	0
9fb2018fba1ad0c094efcf6999c106fa	NA	0	2	0	0	0
b91d7921fa6c442f0e51588d8a317f9f	Chlorophyta	0	2	0	0	0
dabec89cb3337aaa6d36c2011ff07625	NA	0	2	0	0	0
726e5b68acf0b85030b196741416c583	Eukaryota_XX	0	2	0	0	0
634c4bb1ddf7a27e686b0e14b17a29ca	Ciliophora	0	2	0	0	0
17749ea837913e658b7d538ab14268ea	Eukaryota_XX	0	2	0	0	0
289606c0ade2a42b19239402c63d0c89	NA	0	2	0	0	0
521af66f388dcf93f0ec1e9e028d71b5	Cryptophyta	0	2	0	0	0
ad820b6da01ecb5a2c6aa9e2f0503aab	NA	0	2	0	0	0
bc161952940b62df324f617c2514ce28	Eukaryota_XX	0	2	0	0	0
2a37d0fcb3d416a6ee7ae6e3c880c69a	NA	0	2	0	0	0
bee963d844021647356595221c62410c	NA	0	2	0	0	0
0a146d03e922f8cff9499cdba9946939	Cryptophyta	0	2	0	0	0
bf27d7e41ae7e2550b7e178767ee6c89	Streptophyta	0	2	0	0	0
9052e394fe977c9bea4562b72017bc18	Stramenopiles_X	0	2	0	0	0
72ecf84e7183b1afa1a29dba42e1ac4f	NA	0	2	0	0	0
73c85dfe7bde31ada264f2a62cdf460e	NA	0	0	2	0	0
2d3b4289d4392772945f1d569340d5cf	NA	0	0	2	0	0
e7577bf314954efa3c99cefb5689dd57	Metazoa	0	0	2	0	0
d72838af532362f115e93f6d4897cd18	NA	0	0	2	0	0
fa44f163b804e0f080274983ddbb3b80	Eukaryota_XX	0	0	2	0	0
7b6fedbb3816255ec44a205f98d8b877	Dinoflagellata	0	0	2	0	0
03eff1d8e089ff7d97e005b49d230463	NA	0	0	2	0	0
38c44bb07d6c37f3e321ee31ecf01f5c	NA	0	0	2	0	0
1c8a77272dd66075c9c52fe958b743dd	Eukaryota_XX	0	0	2	0	0
6a18c92c79ddd955a5fa81396038f8e8	NA	0	0	2	0	0
c9e0fc3bc16560679b82bf429c3d9953	Stramenopiles_X	0	0	2	0	0
8b9724bcbf23aa67238f0e56001a0852	NA	0	0	2	0	0
c77eed0029c3eb1c0f9bfe9ff109722f	NA	0	0	2	0	0
b039605c6d03bd9ea82b03111d4566e9	Metazoa	0	0	2	0	0
11c87a1f6044df5f21545ad45c783ed9	Eukaryota_XX	0	0	2	0	0
bd52c9da2c903a17c62daf1a563db7e7	Fungi	0	0	2	0	0
fbbf1f143fd2c307e6bcdd2f63b76598	NA	0	0	2	0	0
e4692ff4f2a998653384fea5b0798e1e	Eukaryota_XX	0	0	2	0	0
3fc834ec2562ea9792a162504fae7d13	Eukaryota_XX	0	0	2	0	0
d39377d5c01c115cdfe7d1ac4d18da3e	Amoebozoa_X	0	0	2	0	0
69785ed032fee65efcfdd5f190c47058	NA	0	0	2	0	0
f6918ceabdff2503bb0e4048a2e0f346	NA	0	0	2	0	0
3ca5d4018655d8336e9f3a8ff4fa6367	NA	0	0	2	0	0
c948c796d16bb38e3b4529bc000aea84	NA	0	0	2	0	0
6a519b74e7b5d6737a6d8db30c1bc7e4	NA	0	0	2	0	0
db1a63b2748337a72a036d48bb723625	Dinoflagellata	0	0	2	0	0
2ea3596b5a2ece3108524e91a5118055	NA	0	0	2	0	0
b63f0c8079242fcb6593278a5ccbbf04	Metazoa	0	0	2	0	0

937043d18f373ad6a09052ef6e67513b	NA	0	0	2	0	0
8682c49ef4e64c2690b5cf8da77e735c	NA	0	0	2	0	0
4755b5d3ad0717070b7bf28a96e9a4b1	NA	0	0	2	0	0
1ec025bb92f85c3fda1788ff7bf44597	Ochrophyta	0	0	2	0	0
487194abdd3a1316dd90c4f5650f8bf2	NA	0	0	2	0	0
f2c9882a6160d7c47f6541b0bc66f5a0	Eukaryota_XX	0	0	2	0	0
0e85d23767400642dcf84c80b01b7a77	NA	0	0	2	0	0
f6c3fed3c13fd7c7130bc024498d96fa	Eukaryota_XX	0	0	2	0	0
4cdff7390e600c31ebe5340908c3620c	NA	0	0	2	0	0
d2dc8a9a2631f5a4891b54650f39f8e0	NA	0	0	2	0	0
5ec1c67027b5ff8ea1d5b38aa26dab00	Eukaryota_XX	0	0	2	0	0
93391810b63e3b39297c0ebaedaa2e5c	NA	0	0	2	0	0
9bde7fc29973fdeb9550465623069ef3	Eukaryota_XX	0	0	2	0	0
8abcb9bd4961b6591c72912e8383c628	NA	0	0	2	0	0
44bb6711372aa6bea30efca7d187ed93	Metazoa	0	0	2	0	0
cbba3c9ef0707d27b80bca05d6118ad2	Ochrophyta	0	0	2	0	0
dd197521c58f03271f1da1bde2b56894	Eukaryota_XX	0	0	2	0	0
9104c71fd195a17c613b126bbb26f44f	Eukaryota_XX	0	0	2	0	0
64d449fb4b33ec859be9b0d205200534	Eukaryota_XX	0	0	2	0	0
fb2627eede756a0f1f63317a8834798b	NA	0	0	2	0	0
21028b4083bcf63cab8c74818af63584	Eukaryota_XX	0	0	2	0	0
8dacb1b7911ed694d775cf4ecb097c6c	NA	0	0	2	0	0
20f5493ff89dc984f9fdc7b8c930967d	Eukaryota_XX	0	0	2	0	0
13905f2f84397aaa47baedae0c50d91a	Eukaryota_XX	0	0	2	0	0
a15e7923cb5efe134753606b402fa9c5	Eukaryota_XX	0	0	2	0	0
d37a8a1018b88ef255ee2a38bae92713	NA	0	0	2	0	0
ae8e7b0cea60f6a0a368a8d991d4fc12	Eukaryota_XX	0	0	2	0	0
66139ad5387f8cb42b25103b260f830b	NA	0	0	2	0	0
9f95bc264a56cba8a6c2eca2686cf9da	Metazoa	0	0	2	0	0
8f764fc7edebbcc1af4ed7570318f4b0	Eukaryota_XX	0	0	2	0	0
32bce97a06cfbf1320f352c1c6bc6cfb	NA	0	0	2	0	0
b4f73111960d4f00a97c1e2ee945d5cc	Dinoflagellata	0	0	0	2	0
9025751c989d9b4272edcfa2a55bc129	NA	0	0	0	2	0
50980971e3ad68436c0279c327f68dd6	Stramenopiles_X	0	0	0	2	0
397af5e5f6fab40bc7df4e8579edb227	NA	0	0	0	2	0
f01f08768922f5c42066c836fc9a8718	Ochrophyta	0	0	0	2	0
9e1680c4bd486d41493cce1b81f46e54	Ciliophora	0	0	0	2	0
d7d7b37a1ff5465ca73c09e071834bef	Fungi	0	0	0	2	0
f4aebdfa94418f9ad552be0421e5c7b1	Dinoflagellata	0	0	0	2	0
60771981296934a8bb50338ac1568886	Ochrophyta	0	0	0	2	0
ed0b07c111f05af9bd2864ec6fbe859e	Dinoflagellata	0	0	0	2	0
1327e8088c3dbd3668a9727c59f81ca6	Dinoflagellata	0	0	0	2	0
097000ebe5a284d6e595eb4019893426	NA	0	0	0	2	0
437a1f12bd94e7c308cdf558a9eccee2	Dinoflagellata	0	0	0	2	0
bba52a7e9da05a279434ad4210f85ca9	NA	0	0	0	2	0
e7d637c0058131c34e60fb1dda2bbb49	Eukaryota_XX	0	0	0	2	0

6e8c0cc8983782e75c060ab989873a07	NA	0	0	0	2	0
152bc26765ba2c89b44a82c7a787d080	Eukaryota_XX	0	0	0	2	0
5b5b3c72df3017a003d168dbcf7c8ac8	NA	0	0	0	2	0
9ae019769bfc67c97780548f101fb1d2	Chlorophyta	0	0	0	2	0
84315f62fef6dc2c6db9b033b994232a	Metazoa	0	0	0	2	0
05f7a042ed9e16b6ad2fbf4522c72b2d	Eukaryota_XX	0	0	0	2	0
af2c558682fd124b1d2da97b14a42bec	NA	0	0	0	2	0
64af6e0de5226ce94035c14a35418dd9	NA	0	0	0	2	0
3bf13e3315ef7daf38aa7646061c12ab	Eukaryota_XX	0	0	0	2	0
8346d593220bad2854e1101fd94f6c1a	Eukaryota_XX	0	0	0	2	0
249a1f6ecc8e18c49ee17d2cbd7d357b	Eukaryota_XX	0	0	0	2	0
6f8127fd6836f61131bbb8173c5b446f	Dinoflagellata	0	0	0	2	0
757ea9a55e1046f53d0eed521bd5bd3a	NA	0	0	0	2	0
63cd1159a5a77a843b173bc9967ac98e	NA	0	0	0	2	0
dcb0d12cd554741e3500969a45741512	NA	0	0	0	2	0
2681846a7cae9f27094312709a5aa832	Dinoflagellata	0	0	0	2	0
421cd45797ba5cff7e0a54019d819478	Dinoflagellata	0	0	0	2	0
4771f6dc1664b29344631bf24648ebde	Dinoflagellata	0	0	0	2	0
d49c57c0ca6774d7bea3689599991733	Dinoflagellata	0	0	0	2	0
6dbdf81e17972d766244a1720970eaaa	Eukaryota_XX	0	0	0	2	0
f15352435502bffd34648db28a7bdd50	NA	0	0	0	2	0
4aa7cecfcb8091548786557143122f5c	NA	0	0	0	2	0
21eb22e65d508987445d371489e5e4b9	Dinoflagellata	0	0	0	2	0
2da1b0789b8c53b144c42d53921a7c90	NA	0	0	0	2	0
5f1b31cc0e98a9c8d3f489bdb4811191	Dinoflagellata	0	0	0	2	0
8d0b1dc77a8dd6d648f2132668031b25	Dinoflagellata	0	0	0	2	0
0b5897da267866fe52049bcf54702e3e	Dinoflagellata	0	0	0	2	0
fe7361c86bd65d16885c27a270c2f329	Fungi	0	0	0	2	0
11a5062406196a2c83b85ef62c156b04	Eukaryota_XX	0	0	0	2	0
5b5da624a40e7362525ca8880cf8bd4c	Dinoflagellata	0	0	0	2	0
6960c96ca649e9f2519f720d392b12dc	NA	0	0	0	2	0
7d8c7edc51fc440ef7077bb116403336	NA	0	0	0	2	0
a58efcf616edec4f675aa8a25324192b	NA	0	0	0	2	0
6a003a9666a953d66f103b045c8950a7	Dinoflagellata	0	0	0	2	0
13363acb8c6c1ace78ed45a05c61b4d9	Rhodophyta	0	0	0	2	0
1c5e16aa835ee90f742c2f986c0b40b9	Eukaryota_XX	0	0	0	2	0
dec4e458e10ef136ea4d2fb4575daaef	Ochrophyta	0	0	0	2	0
57c254005f4049919086ad0382a4b075	NA	0	0	0	2	0
f30daecbf80f5c5a4e059b1ef5500a23	Eukaryota_XX	0	0	0	2	0
0eb7a6fe0a53e815daaf16576ae91821	Eukaryota_XX	0	0	0	2	0
610bda39f519d3483b5db43b0d932151	NA	0	0	0	2	0
2b058adfce09197d1ac17fe51a99ec70	Dinoflagellata	0	0	0	2	0
cc66040af71697337b1eaf79e472412a	Metazoa	0	0	0	2	0
560f8105f6947367174a29ef66df9494	Eukaryota_XX	0	0	0	2	0
710528d8049f20bac35ffea77ce6c3c9	Metazoa	0	0	0	2	0
e6394c824931903527f417935083da94	Ochrophyta	0	0	0	2	0

81c2c07fdb31068152eb85f77ed7bceb	Ochrophyta	0	0	0	2	0
42581407ea58121bf1245cdb3befa6b2	Chlorophyta	0	0	0	2	0
8fa02e7db1494d688466c512cffb1921	NA	0	0	0	2	0
3c024d57a495efec59acf5f12923efc9	NA	0	0	0	2	0
ea9b773a27fa2bb44518590b7a877299	Chlorophyta	0	0	0	2	0
071c13b5cfeb274d49db63d6f4a91192	Eukaryota_XX	0	0	0	2	0
0105e42f8275290cb54a394e218a4204	Telonemia	0	0	0	2	0
4028d7fb209a1b0f66caef55d1262dd6	Dinoflagellata	0	0	0	2	0
3cbf5e7cb45fe07f519ea26c95bc7cd9	Stramenopiles_X	0	0	0	2	0
1b96faac9836d2dae1a6c26847cfbfdd	Dinoflagellata	0	0	0	2	0
c2d0cad2b604a61e915c256fb7f3ef82	Fungi	0	0	0	2	0
60371c8a28ced9eea535810dcc204195	Metazoa	0	0	0	2	0
ced35b1befbbd7cf9c74bc7773833d3c	Metazoa	0	0	0	2	0
b0434103eef312272f26208c87715fd6	NA	0	0	0	2	0
04e1dc79fcb8588cb5ed8a26e0deac91	Ochrophyta	0	0	0	2	0
3af85bfffe6cb3d608b9c11fbea04d1e	Fungi	0	0	0	2	0
cd03dc0589b80a76d7cd22f7042741e5	Rhodophyta	0	0	0	2	0
d2defcef55b517e404bcced6a193a990	Dinoflagellata	0	0	0	2	0
a874d6c71727345af723df198969daec	Haptophyta	0	0	0	2	0
22b9216ac107f38fa612a8208d2d055c	Rhodophyta	0	0	0	2	0
63eb28a9e637db0db9f62887e1d34de0	NA	0	0	0	0	2
5a0e85de549b16fbbb74aa711ece28e4	NA	0	0	0	0	2
ddc45a0e511fed46c752d1d754a6c907	NA	0	0	0	0	2
c95464c90ae0a679854b4c62c0d3f595	Eukaryota_XX	0	0	0	0	2
9b21d055f93ca76b6a4a88821302ec51	NA	0	0	0	0	2
463af744d64d8ea68fc0a8b9d0f74db1	Eukaryota_XX	0	0	0	0	2
e620eb5731e91a832cc6471c504f19a8	NA	0	0	0	0	2
39649f2a9cdc2b3cc06a0c557961e28f	NA	0	0	0	0	2
f14b05d3ecda1657ae2f0cea2a57e482	Metamonada	0	0	0	0	2
b8b9575b24210a5ceb884bce669b0566	Eukaryota_XX	0	0	0	0	2
460b84821f44e12d6f378d7c88b34aa5	Eukaryota_XX	0	0	0	0	2
91e6a37355939f60095fafe99126e8ae	NA	0	0	0	0	2
880ae16402da9750bb8b8509fcea0b5c	Eukaryota_XX	0	0	0	0	2
3dd86ccbfb589f11d595f5406a397faf	NA	0	0	0	0	2
fb744658083fe27dfc0f0b7a15004502	NA	0	0	0	0	2
f240bd77e555ac7631a8be6d7815fdb6	NA	0	0	0	0	2
46c76b96b978204d2e39e2c1a54e09ba	NA	0	0	0	0	2
53dd20c070b638011143e3833f9ba6b6	NA	0	0	0	0	2
9aa2a46b383a14a8e9b8578814264427	Eukaryota_XX	0	0	0	0	2
2f53b57a0dbd279052ff45c3ae5ac285	NA	0	0	0	0	2
81276954bc33ad1aae1b9a44145ce0d5	Eukaryota_XX	0	0	0	0	2
650d7a2dc0f2f66a67a2d698ade5a18a	NA	0	0	0	0	2
859fdab6b534cccae74cd3bab8b2248c	NA	0	0	0	0	2
de9ebe63cbec449f051d26c7aaa36e13	Eukaryota_XX	0	0	0	0	2
fca086b27da98c8ee48fc840de152018	Eukaryota_XX	0	0	0	0	2
29c7871e6e308c8796b281a58f659c04	NA	0	0	0	0	2

a1b867d7502db78b21a5f0dcb7d0b88b	Apicomplexa	0	0	0	0	2
b4494ec3878044f6d87cb77d51879263	Eukaryota_XX	0	0	0	0	2
affc8542e07d5b3cfc471674df45c114	NA	0	0	0	0	2
43eeca0892c5423b4709bbd2fb1a7b1a	Eukaryota_XX	0	0	0	0	2
702ab6d8f7f067468cb92bc268e053d6	Dinoflagellata	0	0	0	0	2
b721cbb281be2811c3773da8827956d5	NA	0	0	0	0	2
d739b792aca3d54f541301363eb05478	Eukaryota_XX	0	0	0	0	2
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b662c562a2bcf3c3d0bb5b85a0fff79a	Eukaryota_XX	0	0	0	0	2
97c2006aea0b6fd0d557c49be9b8d9b4	NA	0	0	0	0	2
27d42e8ff24cd69b5e2540a42d15a224	Eukaryota_XX	0	0	0	0	2
9ca411e9268db0dfbb5f94d4f7d7870e	Eukaryota_XX	0	0	0	0	2
14dc81f62e66a9b36c01c8e140fd9e4e	NA	0	0	0	0	2
611560f0dd13bbfb953d879ae59b7143	NA	0	0	0	0	2
18e1ab73adc1a2f091ccaf032b1d1797	NA	0	0	0	0	2
2948a036f3278a147773a2f17f3c48ec	Eukaryota_XX	0	0	0	0	2
aafd4403b6d677ec107d9c0652b487d6	Stramenopiles_X	0	0	0	0	2
cf8cc39238a93c49fd701b37ac6e370a	NA	0	0	0	0	2
af8a635b5a58c8edc92fbf4576adaea1	Eukaryota_XX	0	0	0	0	2
81ba00587ebb768145ae5c5f3624f8a0	Metazoa	0	0	0	0	2
22c755d61ed9119139dbec5b078c47d6	NA	0	0	0	0	2
848e20ad13e146fa74f5a2c415fb2ad9	NA	0	0	0	0	2
48d5512d6d1a3080c71906e0a3269dfc	Eukaryota_XX	0	0	0	0	2
5c5210fdc65d49e79714f065aab87533	Eukaryota_XX	0	0	0	0	2
41ab67919dbe971362b186c4ce24d2c8	NA	0	0	0	0	2
eb1413f99061d774188f6a9b00045aab	Eukaryota_XX	0	0	0	0	2
de1a824214777f299035d0dc6135bb85	NA	0	0	0	0	2
7973d50d21aa6f4ac24a0d8adc41215f	Eukaryota_XX	0	0	0	0	2
b0cad81e19a5b57d7499387336ba3a0d	Eukaryota_XX	0	0	0	0	2
6a5d74836bbfd027ef5cc6caeee2be73	Eukaryota_XX	0	0	0	0	2
1301da02a65c2e90d39929c5f5a6c733	NA	0	0	0	0	2
ecfb135ac7fefe0318ae789e8c6df478	Eukaryota_XX	0	0	0	0	2
3d9cff82e80ec52b2fbc2ac10efddc7b	Eukaryota_XX	0	0	0	0	2
d94ac1d76ee73aab76f975a79b9276b4	NA	0	0	0	0	2
cc64ab339dfcb5aa1d46a6d445fdf752	Eukaryota_XX	0	0	0	0	2
fe1a012945c1febfac26ecf1cac5c97e	NA	0	0	0	0	2
e80e02cf103825e564adb305508e3055	NA	0	0	0	0	2
d59d76afca914c2056b3b5eac4e147ba	Dinoflagellata	0	0	0	0	2
198388abcbc30569d6272f4a45cdcb24	NA	0	0	0	0	2
01542ece08e0d16a7f3b34b55119bdaf	Eukaryota_XX	0	0	0	0	2
ad0d147f40e003ed4b43a6b40de7a4f7	NA	0	0	0	0	2
297f88dd2811ddda5597a8737dbd3675	NA	0	0	0	0	2
26d4ae0539a3e76c4e50694fa0567264	NA	0	0	0	0	2
def0c72c1f17df7d6f858137e4c17f60	Eukaryota_XX	0	0	0	0	2
96b8cce0d9c71fd9ef3755fecf707924	NA	0	0	0	0	2

2df3effe4ff38d388f9f5a9820a332ed	NA	0	0	0	0	2
d0cba414a7de760087991a3ad1b5f36f	NA	0	0	0	0	2
7e4c9033a38b9c42cf6c5cf4ec41c241	NA	0	0	0	0	2
0cd2a30d6253efb05f3d8ff00de362aa	Eukaryota_XX	0	0	0	0	2
5cab90236bd977ba09b755825096eac4	NA	0	0	0	0	2
89c4e0a3ff0539c963e487621f0d772b	NA	0	0	0	0	2
5f8aebffd121d65a7d55ecefb0d2672a	Dinoflagellata	0	0	0	0	2
181c134073b1bc6e23f0b1fe2b3f32c3	NA	0	0	0	0	2
9a32e5571892b26882eba70e32a6ae4b	Eukaryota_XX	0	0	0	0	2
a56cb894befee5a9b96f11c5cf18ac4f	Eukaryota_XX	0	0	0	0	2
a53e49a2aef4c576dc5577c640d9b460	NA	0	0	0	0	2
c2e9164f7c8af1e905190ff717075042	NA	0	0	0	0	2
9383ddaca9b645873c8162cbb01bceef	NA	0	0	0	0	2
f7cb612b7228d9dff6df6f50c6003013	NA	0	0	0	0	2
8d4650e5b3276988924f1c0777c6cd2f	Eukaryota_XX	0	0	0	0	2
89c2630088d3dcf84f738afe16891176	Fungi	0	0	0	0	2
f7f4154b61e213d795af43f70c5e9063	Eukaryota_XX	0	0	0	0	2
8707e6c6a08c9cd64c8fff7d0d1d9d5d	Eukaryota_XX	0	0	0	0	2
295013221988619ee20adc585f019797	NA	0	0	0	0	2
763b435bb10beb53a0ca06bb0653dbae	Eukaryota_XX	0	0	0	0	2
6804ac19cba8254eed73cb632370a628	Ochrophyta	0	0	0	0	2
ec793d54cb38031c7f5ffc398ba0672d	Metazoa	0	0	0	0	2
9d12de03c1e0ac6955d7c19ec7f4270d	NA	0	0	0	0	2
3a806d9df5e89f8d2c563e6fa5468112	NA	0	0	0	0	2
524530f472de4ddc693b9bd00752c91c	Ochrophyta	0	0	0	0	2
aa3c563018d39102f6cf55872a74382f	NA	0	0	0	0	2
cfd7846dba455def763352d1dfea56f9	NA	0	0	0	0	2
6111ebb6f0ab2495aa9dc1960fd1075f	NA	0	0	0	0	2
a76e319c85b631de48e46a7b7628c995	Eukaryota_XX	0	0	0	0	2
e5c5257e4a55802d574ff7d2d9453e9a	Eukaryota_XX	0	0	0	0	2
47ff03492810265c6ecedd9b8c5b1db7	NA	0	0	0	0	2
2e2563cc785142b1e3f43faf91ced65c	Eukaryota_XX	0	0	0	0	2
6a05b407ba910e5b4df186207244d45b	Eukaryota_XX	0	0	0	0	2
35b11114235c82d204561844bc07ff56	NA	0	0	0	0	2
7e8c288f863fdb076aed5e24eb2833b0	NA	0	0	0	0	2
511f2723a46e4c823f236aab6928c613	Eukaryota_XX	0	0	0	0	2
847a18cc7e16e28b9af75e973ab37762	Metazoa	0	0	0	0	2
781bbbee3fddc2c604aec2e93076f0a4	Dinoflagellata	0	0	0	0	2
1c627cb1985a33b7d9b633e7a0a31245	NA	0	0	0	0	2
b754473abc12e06d963b6fd6fbbe2b05	Eukaryota_XX	0	0	0	0	2
fd94d45dd64ce7cd6a17230f3f63122c	NA	0	0	0	0	2
cf20da0e01bd9a259b5e012cc4032a3b	NA	0	0	0	0	2
707e3be8ec592ed8474d9f918d9d6752	NA	0	0	0	0	2
c39543093be38c818cad1a786f345c76	NA	0	0	0	0	2
91894650006744987 59de637184bbb4a	NA	0	0	0	0	2
be98432888ee512e191b463530fe8830	NA	0	0	0	0	2

cc3412027ef83aaa2bd28171ae6a1b00	Dinoflagellata	0	0	0	0	2
a550daf2d2953c47dfc380bdef7fc257	Eukaryota_XX	0	0	0	0	2
d4b2915725e0e9dd55735c6437bfbc41	Fungi	0	0	0	0	2
8fd90865b7f7f94cb77c8935d41504b1	NA	0	0	0	0	2
0ad3b147657552be7b2b565e95802c09	Eukaryota_XX	0	0	0	0	2
74cb97f5e40a384e4b2f0270c9a6a919	NA	0	0	0	0	2
0ffe7cfd8706e09089b87da35d0f013f	Metazoa	0	0	0	0	2
8d32be93e3eb754d31c7cce7fc7665d8	Eukaryota_XX	0	0	0	0	2
dad19a45ced589afb1ad713781d64bff	Ochrophyta	0	0	0	0	2
d72ae226ea1d0ceaadb5d524ee2e83b6	Eukaryota_XX	0	0	0	0	2
71176f7544a2d6f1b4b9b3e641b7a624	NA	0	0	0	0	2
fdb77b931004ef0377098cbe2657fd7e	NA	0	0	0	0	2
2a9fdbcb0ae718baafee046ba603e209	Ochrophyta	0	0	0	0	2
4518f8596aba0b1c6778e3d908dab2b4	NA	0	0	0	0	2
90f97bf8435f5a98836581ef270872bf	Eukaryota_XX	0	0	0	0	2
e700b583f90889a66ace34b094cdfcbe	NA	0	0	0	0	2
8d9fbb1985cd6adeee66d6e26ec15c85	NA	0	0	0	0	2
ab30cc84194168c47b1ae537fd6449a6	Eukaryota_XX	0	0	0	0	2
e3d2a748a99510289a481e82baa3e405	NA	0	0	0	0	2
061e5eedaf9b37fe6e6e0d9da026d7bf	NA	0	0	0	0	2
ee17ea0ae92f52887b966deabda18a74	NA	0	0	0	0	2
193e41e6a4cca7514088e4618ba5701b	Eukaryota_XX	0	0	0	0	2
6d7c2bb45eb2bed7c1bbcc37b4ad461c	Eukaryota_XX	0	0	0	0	2
58233def916c55d75e0299a8769901aa	NA	0	0	0	0	2
d8c9f5ade908cd43cf1d3df211dab30e	Eukaryota_XX	0	0	0	0	2
221f7ddbe9de6cc07d46171ff2a075cc	Eukaryota_XX	0	0	0	0	2
59084ebde6fb15973b411ff4a72f83e3	Eukaryota_XX	0	0	0	0	2
214a6bd86ae9373b6afa139cba6f5d3b	Eukaryota_XX	0	0	0	0	2
898f8add31d43d66d123e0c4d4721d44	NA	0	0	0	0	2
299193dbef9bb5f128deec7e3f2b1dcc	Eukaryota_XX	0	0	0	0	2
a8527156edd93bd5e335bae5cb25e025	NA	0	0	0	0	2
e41a162d3d0f43f0e46ffc30610bf5da	Stramenopiles_X	0	0	0	0	2
56dfd511b614b320c19805dcd2e0ab4b	Choanoflagellida	0	0	0	0	2
3374b6e20cf9e430c28e519a2b9932b1	Ochrophyta	0	0	0	0	2
4182bd1b1460ea6a3bd2b9c4f9254dc6	NA	0	0	0	0	2
d62b0dcb9a14e65c2e8ec7d373b3478d	Eukaryota_XX	0	0	0	0	2
034e9288e9bccea82bda3d47c72cb39d	NA	0	0	0	0	2
19745a07d4ccc4e1273ea27256b834df	NA	0	0	0	0	2
6d8b21f43ea86a84e176d551d608fcff	Eukaryota_XX	0	0	0	0	2
fd4e209e09a373f2aec7641b9d207295	NA	0	0	0	0	2
4380465aec6705eedc799966c85e846e	Eukaryota_XX	0	0	0	0	2
461d56aa47521f6647bb2992128a7810	Dinoflagellata	0	0	0	0	2
7c9c316504316c2566b66baf85fc9af2	NA	0	0	0	0	2
6c31b11a79e4a13976fac7fc930c438b	NA	0	0	0	0	2
76ef9cb758ceb76ab641fe23c735f8f6	Dinoflagellata	0	0	0	0	2
b414e8902596f5eb0c2442d2ca2309a1	Eukaryota_XX	0	0	0	0	2

21142a2059b6d90db918cf0df87ad4ed	Eukaryota_XX	0	0	0	0	2
3d04381090b90e7da1c3cc517f813e2d	NA	0	0	0	0	2
58587ed4f67791bfab680baabae5fcff	Ochrophyta	0	0	0	0	2
2bd8e7cc14a23be2052970b4efc472c6	Eukaryota_XX	0	0	0	0	2
d981359ba5dde7ec309710265ce9aa99	NA	0	0	0	0	2
dd3188dbbd68ecdd8ab78e9a1e91beaa	Ochrophyta	0	0	0	0	0
f48de0fe703d9da7ea0a7581f1f9d021	Ochrophyta	0	0	0	0	0
76532e65ecffd159736c5156dfebf751	Eukaryota_XX	0	0	0	0	0
cf2f8bc5ef989644e8e57a18c386f8d9	Eukaryota_XX	0	0	0	0	0
fc8ebc1fec03ea3bd64dacab47930a1f	NA	0	0	0	0	0
484ddaf0f85398e71f53b31865a60432	NA	0	0	0	0	0
a44bc6b1c86fe0d19ab619d4ca7048dd	Dinoflagellata	0	0	0	0	0
ad25a9153f30cfd014646529fb8430ad	NA	0	0	0	0	0
2ec747cfda819998af1a6c2eabeb38b0	Eukaryota_XX	0	0	0	0	0
8fcc22c09308245cabd251fbd28f9d40	NA	0	0	0	0	0
05c3730589345230d74da9cc5e6633d0	Ochrophyta	0	0	0	0	0
2f8cf94d61f31d7d727b219f567262d7	Eukaryota_XX	0	0	0	0	0
03988e7f09818a149df0f02f1734ddf7	Eukaryota_XX	0	0	0	0	0
c9b7d4a6f300b96bfbade08d4f84992f	NA	0	0	0	0	0
38401e6e59333eeb9b63695c350d9dde	NA	0	0	0	0	0
096b49443dd9d1f059253eac18435280	Fungi	0	0	0	0	0
9c84ec6554d078c34f6cf88abd8ad29d	Eukaryota_XX	0	0	0	0	0
c53e670dfff1d5ae6a3e603b08f7c90d	Dinoflagellata	0	0	0	0	0
e899f85111130f808ccd8146b170341b	NA	0	0	0	0	0
6ae5d64453151bddd20a4af5515923ce	Dinoflagellata	0	0	0	0	0
abeb1afda7b11fce065817ad9ec6cfa7	Ochrophyta	0	0	0	0	0
924e83556a01fcb1b4f52237d31bb739	Metazoa	0	0	0	0	0
7bed380b4c90f81c6eed37e589a139d0	NA	0	0	0	0	0
21961667fe6c8e4d62cfe2e70418cb1c	Eukaryota_XX	0	0	0	0	0
2e3e90edab243c4eb72c2afc0d1cdf23	Eukaryota_XX	0	0	0	0	0
25ba6a66eeb8a4ca7a8fdb119fca0c94	Ochrophyta	0	0	0	0	0
ba912708ecd39ee430b21fdb8d71f9f6	NA	0	0	0	0	0
60013e71ed2cd1bb15e0e7e36d2671aa	NA	0	0	0	0	0
86df80703908995c5c5be2861fed182d	Conosa	0	0	0	0	0
cce0fffe7a908a4fe055799b97367056	Eukaryota_XX	0	0	0	0	0
66f5a35ba603cf8cf55bfef7ea02f2af	NA	0	0	0	0	0
f24bb829f8d6061bfac9a24b6f966b4d	Eukaryota_XX	0	0	0	0	0
7468f508a52f7efb6ceec3a4f300818e	NA	0	0	0	0	0
e725196e65c624c0b65cea282d2518ac	Dinoflagellata	0	0	0	0	0
5494b348f953c1daad6c4c89aefb5aab	Eukaryota_XX	0	0	0	0	0
f5138f4c8cf6bb65fc7974e39b3a1e69	NA	0	0	0	0	0
48983d46a31a2f3f6e19e8b9f5a2d554	NA	0	0	0	0	0
1235fb42fe423f1bda4d0fc3f92ae5b5	Cercozoa	0	0	0	0	0
56da82370618846eb40bdb01031274b5	Eukaryota_XX	0	0	0	0	0
76b1647b9f9587db74d7c6b1bec1064e	NA	0	0	0	0	0
f39dfb491559f9507e10a5ce903ad1ca	Eukaryota_XX	0	0	0	0	0

2f57e2581f5e05d9ad27af753b362953	NA	0	0	0	0	0
7892787696acabec4f8024475397692c	NA	0	0	0	0	0
3ab3e2ab848a7e88afd304ea3dad1b2b	NA	0	0	0	0	0
486ae157b9de445fe9397e63232781c7	NA	0	0	0	0	0
c772316733e4e0e4a2fad89f6475ebec	Metazoa	0	0	0	0	0
78354aef4d102ea514e4c0892b767bde	NA	0	0	0	0	0
5b2830931c69b4c4980c56160618d984	Eukaryota_XX	0	0	0	0	0
1fdaf0da38088cb56685788c95ec3d1a	Ochrophyta	0	0	0	0	0
40f4b209d972559cda1cd87e08574c09	NA	0	0	0	0	0
043bb41bb7cb9027668c08ac2eaf5ad4	Eukaryota_XX	0	0	0	0	0
d3fbe8e4011f49385c68f89291c76308	NA	0	0	0	0	0
e10bf10bfa60910d736f8349e2ef936c	NA	0	0	0	0	0
b9ecc643d72def435530df2e62b3642f	Eukaryota_XX	0	0	0	0	0
96db27821552ccce86f7a87ae22e7af2	Eukaryota_XX	0	0	0	0	0
c51fb98d913e406e1ea3e35aaad6e896	Cercozoa	0	0	0	0	0
121c9b4cb11d9551634eb414e75e280e	Eukaryota_XX	0	0	0	0	0
9a20968bc6eb41a3b2d668f2a478886b	Eukaryota_XX	0	0	0	0	0
c2a3653d41ca610517584681b3497b98	Stramenopiles_X	0	0	0	0	0
028fd8a2eaafdb31d17af24072a0fe4c	NA	0	0	0	0	0
fca6099be065aab7d934a36d12e48be3	Metazoa	0	0	0	0	0
35b491ab83f4e187500df46962bc51ee	Dinoflagellata	0	0	0	0	0
9d2cbabde900468db088528a3a4d1e2e	Metazoa	0	0	0	0	0
69ff203da51960ad0bb0634ef09a11da	Eukaryota_XX	0	0	0	0	0
af6e3d959a83a4ac7d5ae5336a622a90	Dinoflagellata	0	0	0	0	0
0cb2c2e8a932065d5eb986e16aaa1521	NA	0	0	0	0	0
bdea3ffe65acc1c3273ca82d54251731	Eukaryota_XX	0	0	0	0	0
87b192bfa9b290eb5d33704e0f505c81	NA	0	0	0	0	0
beef190cb4ee6ade0506a6db9221e455	Ochrophyta	0	0	0	0	0
6d5cf586694946b6bb869e42e392e5b3	NA	0	0	0	0	0
177473705f7ba86f80efc6721c428fd6	NA	0	0	0	0	0
fd45a75f43770433e0286f2d0beeca0d	Eukaryota_XX	0	0	0	0	0
9e2af749aac3581bc8ae81bedc4c2b5f	NA	0	0	0	0	0
29de95f0155a78a55d147fb538fc5b88	NA	0	0	0	0	0
05e60073771ce1723ef375db2f2bc10c	NA	0	0	0	0	0
dff7856cb35bf30536fea9062ff86cf4	NA	0	0	0	0	0
e8989aaa56f7a2f91aa9ba4f79f817bb	NA	0	0	0	0	0
0f053c643049b0b9e834490fa86e2dd5	Haptophyta	0	0	0	0	0
6ef83b435a81b4266125b33a4ed3ea97	NA	0	0	0	0	0
0cb693b8723f3a0139af654cfeae6816	Ochrophyta	0	0	0	0	0
8358a46e40b105a46635441c2971eba1	NA	0	0	0	0	0
e8a4a51562e8095dd1e84ab8504282a7	Foraminifera	0	0	0	0	0
9e25ac5567346b109e418c74ff8eb411	NA	0	0	0	0	0
8e7ad6fb6ce178db676915b27b90266d	NA	0	0	0	0	0
8f4a01e3a8af9b045f364e6892ed20cb	Dinoflagellata	0	0	0	0	0
c8520d2c6558622bed1ce802473bda63	Ochrophyta	0	0	0	0	0
f6681e2d1b92ee93df301cc258922796	NA	0	0	0	0	0

51ec6d5ce05fcc746d38a6a73f783581	NA	0	0	0	0	0
49feb5dbb87b136faf781a07e82f2b97	NA	0	0	0	0	0
6ac1185c25116381d29b46f261201621	Ochrophyta	1	0	0	0	0
21efb271d049f92478b3fdcc63646c19	Ochrophyta	1	0	0	0	0
8e19617d12ea5ef7d5c47045826d44d1	Ochrophyta	1	0	0	0	0
bc2112921c6348a56bf0d5d424929abb	Dinoflagellata	1	0	0	0	0
aa1d786fa8e7dfe771730e2477ff14d8	Ochrophyta	1	0	0	0	0
699e7b980be63da2ae67ff69747d6cb4	Ochrophyta	1	0	0	0	0
7210da124dc17e3af861753903cbafb1	NA	1	0	0	0	0
88709776433c7491c3cdff358eba3977	Ochrophyta	1	0	0	0	0
d720298b483aad6d91ff600009bc77cf	Ochrophyta	1	0	0	0	0
08868f0f564fb1d7f22939a916b30862	Ochrophyta	0	1	0	0	0
ad2387b34527ee8fd1acd6705f04eff1	Ochrophyta	0	1	0	0	0
4b203d746df6c1de07a42ed8a8005511	Ochrophyta	0	1	0	0	0
0743214b3966e1b5e2fb215ae1e0397c	Picozoa	0	1	0	0	0
4eb746c85206870c47bbc1c40edfc045	Ochrophyta	0	1	0	0	0
aa3bb45c932a58dac3651d27b5aa173c	Ochrophyta	0	1	0	0	0
d945a2f97bc1ca56a93977d50b316575	Dinoflagellata	0	1	0	0	0
b45832e0da5b9b2d047a540462a2fc0b	Ochrophyta	0	1	0	0	0
7b6c86f07de39bc2092c0d1960dc127f	Ochrophyta	0	1	0	0	0
50b9aea583673dedb9860426e39289ba	Ochrophyta	0	1	0	0	0
9a8524daeffbe59e5969b5f3d51a7ded	Ochrophyta	0	1	0	0	0
5170cbbf1927806290ebeb1824d117aa	Ochrophyta	0	1	0	0	0
5fe13e0a2a06e3656086554d08885199	Ochrophyta	0	1	0	0	0
129abf2ac996dc840927ccea4d65eac9	Ochrophyta	0	1	0	0	0
7d38163f16537abc48525d665ab32c8a	Dinoflagellata	0	1	0	0	0
a81ecd37338489a00c3915040fc51e09	Ochrophyta	0	1	0	0	0
1f29a86dac296e91b7208f6eede34213	Choanoflagellida	0	0	1	0	0
03db75731d036c4d55a6413fdc245886	Ochrophyta	0	0	1	0	0
6702da3926482b2646a6f60e89cbd3f0	Ochrophyta	0	0	1	0	0
9c1fb1c24b920b781bc6506927b138b5	Ochrophyta	0	0	1	0	0
715d896e2468791fb12c2b13427a8d6d	NA	0	0	1	0	0
a86a317beb08fd9b8456b681d94c7731	Ochrophyta	0	0	0	1	0
e860ea3a920ee87c4c014f2bfebcec67	Dinoflagellata	0	0	0	1	0
8a05d91e9f919ab0e8cb51294c2dbcd5	Ochrophyta	0	0	0	1	0
d851c85d8e441bf6c8ebddbe572b7726	Metazoa	0	0	0	1	0
30e9edd4d028658b197139b9f9b420b8	Dinoflagellata	0	0	0	1	0
74cf602487695b35c975649b768ecf03	Ochrophyta	0	0	0	1	0
559c3c0eaedeee97bd7b0e8591796625	Ochrophyta	0	0	0	1	0
1c327d52dec09459510d9d0bee6110b4	Dinoflagellata	0	0	0	1	0
40ce406a371d058390f48b420a7e14c3	Dinoflagellata	0	0	0	1	0
5a4dd01ad84c7a9578bada59c47ee267	Ochrophyta	0	0	0	1	0
7289e28627416979d9c75dda39868b19	Ochrophyta	0	0	0	1	0
86217634d059ce14659a901096abf1fc	Ochrophyta	0	0	0	1	0
dac1517c7ae6854340fd60b8c3f00c8b	Metazoa	0	0	0	1	0
c77a019899139c530941f3c9fcd6a9dc	Ochrophyta	0	0	0	1	0

6302914ce36bc58f1a34200f8a11c793	NA	0	0	0	1	0	
a692291b43bbab28d1bd3ef796f97d3f	Metazoa	0	0	0	1	0	
4c05532f5c3a785c763da115646d0eff	Ochrophyta	0	0	0	1	0	
529e056cf73b62d2dfed844c16b81a24	Ochrophyta	0	0	0	1	0	
f5ba31c910f849d4968257d0d659ec10	Metazoa	0	0	0	1	0	
f32281aa96bc368a1c87381b915a4e8b	Stramenopiles_X	0	0	0	1	0	
45bb6787bdddb73f6efe1e70fcf71d17	Dinoflagellata	0	0	0	1	0	
970a8ef9ab2808b97548acb5dabaa667	Ochrophyta	0	0	0	1	0	
0bed0b1df6d6a9545f131029a31b5c6e	Ochrophyta	0	0	0	0	1	
558528c7f473bcac72a2673bafebafae	Ochrophyta	0	0	0	0	1	
e585961c64b7abd9e5f566482a635c92	Ochrophyta	0	0	0	0	1	
d8a3fe8283e796009d2e23c83e64ca2b	Ochrophyta	0	0	0	0	1	
8678a2c82fce28c7a8482ae4eaec7249	Ochrophyta	0	0	0	0	1	
9bc68908dca23f1d419a7381b305430c	Ochrophyta	0	0	0	0	1	
4329fc3bf6afe1605408d269350f4298	Ochrophyta	0	0	0	0	1	
84ec61422ea0f8c1cdfe705fb513d761	Ochrophyta	0	0	0	0	1	
170d410784bd031a95fa5b08598557da	Ochrophyta	0	0	0	0	1	
280712cfb82eef7623b9d0f85314c36f	Ochrophyta	0	0	0	0	1	
d2664b53bde1598266259e0447b6452a	Ochrophyta	0	0	0	0	1	
9c8c9ca14eb3ffb2afa27d7f4b6f73b7	Dinoflagellata	0	0	0	0	0	
ac0a298aaab0be966004260a5148d297	Dinoflagellata	0	0	0	0	0	
ca7957af36bfe4936459f31a7be1581e	Dinoflagellata	0	0	0	0	0	
758eed62d244074564a303771bafbabf	Metazoa	0	0	0	0	0	
e9d5c7bc55b50842d30555fef2ef780e	Ochrophyta	0	0	0	0	0	
20363129aab6f25b337256c71ec3db51	Ochrophyta	0	0	0	0	0	
6305e24d28c9d298e86ec6412bc2ae12	Fungi	0	0	0	0	0	
c5c6ed62ed98f935ba0f148c61e6b3a4	Dinoflagellata	0	0	0	0	0	
bbfe21b06e744129e0542425ce838d8b	Fungi	0	0	0	0	0	
f1c0bf2dcde077ec7c61f8adeda65c17	Ochrophyta	0	0	0	0	0	
f21bdafaec86f8bc7b66ab08f3f82dda	Dinoflagellata	0	0	0	0	0	
d4ac134b983a73e23912449f2b2112bd	Dinoflagellata	0	0	0	0	0	
853ad7659d6c92a51467b06dca918325	Ochrophyta	0	0	0	0	0	
d15c644bd6af6a94d91bb30d682da7a7	Dinoflagellata	0	0	0	0	0	
c362c8491019efba9a27344889aa64d4	Dinoflagellata	0	0	0	0	0	
		108746	84477	1078014	974557	152744	2897

Table S4

LC-MS Method			
Time	Flow Rate (mL/min)	% A (Water: FA: NH4OH 500:0.075:0.3, V/V/V)	%B (Acetonitrile: water: FA 700:300:0.1, V/V/V)
Initial	0.4	2	98
5	0.4	2	98
7.5	0.4	50	50
9	0.5	50	50
9.5	0.5	2	98
10	0.8	2	98
10.6	0.8	2	98
11	0.4	2	98

Probe Name	Accession #	Class Name	Analyte Type	% Samples above Threshold	abanti data_01A_01A.RCC
					abanti data_01A_01A.RCC
sxtA	KM100455.1	Endogenous	mRNA	100	22
sxtA1_1	JF343242.1	Endogenous	mRNA	100	885
sxtA1_2	JF343240.1	Endogenous	mRNA	100	178
sxtA4	KF164508.1	Endogenous	mRNA	100	59
sxtB_1	JV310097.1	Endogenous	mRNA	100	279
sxtB_2	JV310182.1	Endogenous	mRNA	100	17
sxtD	JV310070.1	Endogenous	mRNA	50	1
sxtG_1	JV310276.1	Endogenous	mRNA	100	10638
sxtG_2	JX995116.1	Endogenous	mRNA	100	35
sxtH/T_1	GAIQ01040808.1	Endogenous	mRNA	83.33	4
sxtH/T_2	GAJB01054897.1	Endogenous	mRNA	87.5	5
sxtH/T_3	GAIV01072833.1	Endogenous	mRNA	100	47
sxtH/T_4	JV310068.1	Endogenous	mRNA	100	860
sxtI_1	JV310148.1	Endogenous	mRNA	100	6123
sxtI_2	GAIQ01063083.1	Endogenous	mRNA	100	19042
sxtI_3	JV310099.1	Endogenous	mRNA	100	220
sxtU_1	JV310191.1	Endogenous	mRNA	100	638
sxtU_2	GAIU01040831.1	Endogenous	mRNA	100	111
CPH	GAJB01040363.1	Housekeeping	mRNA	100	339
DYN	GAIV01030735.1	Housekeeping	mRNA	100	1096
ETIF	GAIQ01034969.1	Housekeeping	mRNA	100	3686
GTEF	GAIT01059349.1	Housekeeping	mRNA	100	794
ICDH	GAJG01016278_ICDH.1	Housekeeping	mRNA	100	3465
IPP	GAJG01016278_IPP.1	Housekeeping	mRNA	79.17	4
NEG_A	ERCC_00096.1	Negative	SYSTEM	66.67	10
NEG_B	ERCC_00041.1	Negative	SYSTEM	75	11
NEG_C	ERCC_00019.1	Negative	SYSTEM	66.67	10
NEG_D	ERCC_00076.1	Negative	SYSTEM	100	12
NEG_E	ERCC_00098.1	Negative	SYSTEM	66.67	10
NEG_F	ERCC_00126.1	Negative	SYSTEM	100	12
NEG_G	ERCC_00144.1	Negative	SYSTEM	66.67	10
NEG_H	ERCC_00154.1	Negative	SYSTEM	100	12
POS_A	ERCC_00117.1	Positive	SYSTEM	100	30260
POS_B	ERCC_00112.1	Positive	SYSTEM	100	7565
POS_C	ERCC_00002.1	Positive	SYSTEM	100	1891
POS_D	ERCC_00092.1	Positive	SYSTEM	100	473
POS_E	ERCC_00035.1	Positive	SYSTEM	100	118
POS_F	ERCC_00034.1	Positive	SYSTEM	100	30

abanti data_01B_01B.RCC	abanti data_02A_02A.RCC	abanti data_02B_02B.RCC	abanti data_03A_03A.RCC
abanti data_01B_01B.RCC	abanti data_02A_02A.RCC	abanti data_02B_02B.RCC	abanti data_03A_03A.RCC

18	3474	22	183
1033	541	681	701
208	8480	136	357
69	2631	81	169
326	286	189	259
11	10	14	36
3	1	1	1
12420	3962	7905	7794
41	10	24	17
1	2	7	5
3	1	2	7
55	25	43	40
1004	192	492	381
7149	2962	3936	4507
22232	5565	12824	10581
257	121	183	152
745	369	461	601
130	58	132	82
395	34	311	198
1279	220	754	398
4304	3844	2552	4964
928	350	641	526
4046	1018	2017	2057
2	9	1	4
1	5	1	4
1	6	1	5
1	5	1	4
2	7	2	6
1	5	1	4
2	7	2	6
1	5	1	4
2	7	2	6
27240	32148	26108	31188
6810	8037	6527	7797
1702	2009	1632	1949
426	502	408	487
106	126	102	122
27	31	25	30

abanti data_03B_03B.RCC	abanti data_04A_04A.RCC	abanti data_04B_04B.RCC	abanti data_05A_05A.RCC
abanti data_03B_03B.RCC	abanti data_04A_04A.RCC	abanti data_04B_04B.RCC	abanti data_05A_05A.RCC
4747	84	2052	3495
1035	762	503	870
15957	217	7099	10085
5901	54	2734	5631
547	180	251	373
18	8	10	29
3	2	1	3
7612	8894	3398	5170
19	15	7	8
2	2	2	6
3	3	1	4
71	18	27	54
504	379	205	455
5938	4849	2658	4111
11747	11316	5286	10423
332	136	144	195
669	477	337	501
120	46	45	117
80	181	47	91
477	407	200	720
7103	4131	3760	3399
734	356	362	721
2361	1718	1045	2150
4	1	1	4
2	1	1	10
3	2	1	11
2	1	1	10
4	3	2	12
2	1	1	10
4	3	2	12
2	1	1	10
4	3	2	12
31084	23632	25320	30424
7771	5908	6330	7606
1943	1477	1582	1902
486	369	396	475
121	92	99	119
30	23	25	30

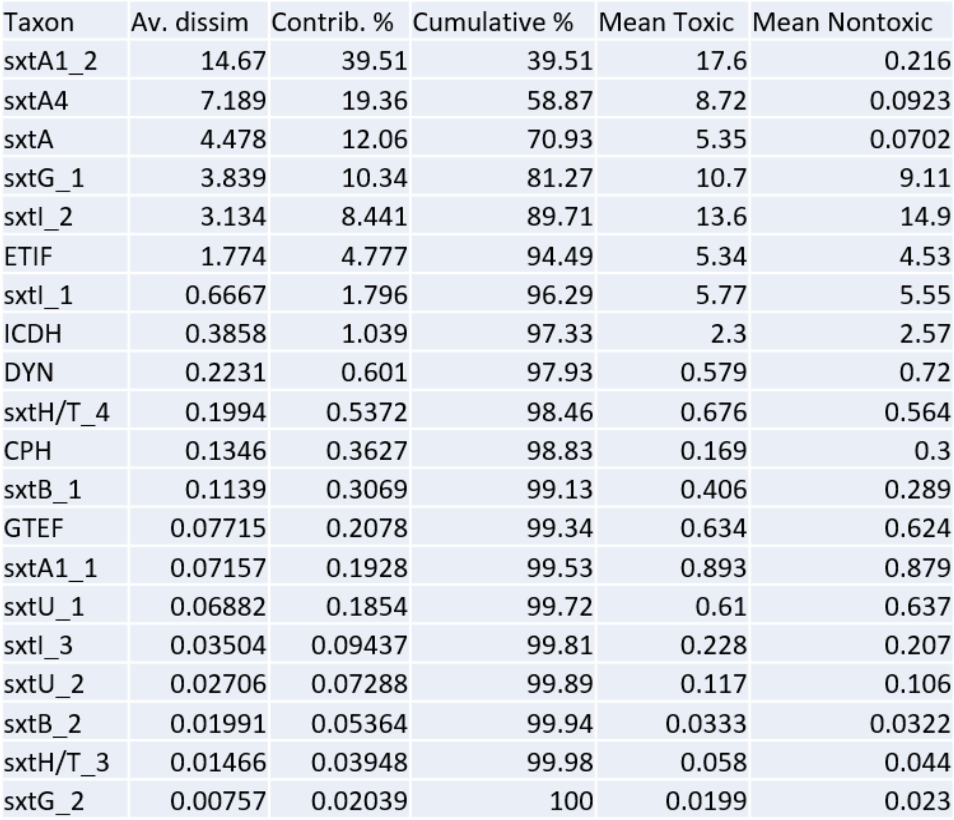
abanti data_05B_05B.RCC abanti data_05B_05B.RCC	abanti data_06A_06A.RCC abanti data_06A_06A.RCC	abanti data_06B_06B.RCC abanti data_06B_06B.RCC	abanti data_07A_07A.RCC abanti data_07A_07A.RCC
57	41	4488	188
1192	646	775	1252
230	212	19317	337
138	46	11850	146
459	249	328	392
78	9	43	75
1	1	1	3
7880	8466	12869	10806
24	10	23	30
2	1	12	7
2	4	5	11
91	26	74	60
674	344	833	664
6443	4880	5510	6880
18307	12195	15366	16628
292	162	189	243
797	425	533	970
198	61	126	129
587	153	234	317
1182	357	650	872
3989	2813	3562	7371
833	393	570	760
3056	1754	2207	3463
1	4	4	4
1	4	3	4
1	5	4	5
1	4	3	4
2	6	5	6
1	4	3	4
2	6	5	6
1	4	3	4
2	6	5	6
24384	26876	26396	29656
6096	6719	6599	7414
1524	1680	1650	1854
381	420	412	463
95	105	103	116
24	26	26	29

abanti data_07B_07B.RCC	abanti data_08A_08A.RCC	abanti data_08B_08B.RCC	abanti data_09A_09A.RCC
abanti data_07B_07B.RCC	abanti data_08A_08A.RCC	abanti data_08B_08B.RCC	abanti data_09A_09A.RCC
75	8569	27	5603
756	816	830	729
181	25199	153	18165
117	12795	64	10933
268	344	247	235
38	53	12	52
4	9	1	8
8447	17563	9667	12648
18	28	26	21
1	6	3	4
2	4	4	8
44	51	27	44
495	1074	579	805
5617	6689	5355	5075
12933	17876	14792	13926
190	182	210	157
688	625	548	473
88	128	84	136
290	247	290	229
402	644	672	639
8287	5005	3716	3161
595	663	588	459
2172	2649	2725	2238
4	16	2	12
2	16	1	12
3	17	1	13
2	16	1	12
4	18	2	14
2	16	1	12
4	18	2	14
2	16	1	12
4	18	2	14
28220	25868	25116	24212
7055	6467	6279	6053
1764	1617	1570	1513
441	404	392	378
110	101	98	95
28	25	25	24

abanti data_09B_09B.RCC abanti data_09B_09B.RCC	abanti data_10A_10A.RCC abanti data_10A_10A.RCC	abanti data_10B_10B.RCC abanti data_10B_10B.RCC	abanti data_11A_11A.RCC abanti data_11A_11A.RCC
2068	107	2534	7421
500	1511	841	774
7394	265	9351	22151
3052	137	5648	8900
273	529	400	308
7	112	15	45
1	3	1	8
4195	9382	5275	13607
8	23	13	17
5	5	5	8
1	6	6	4
38	86	55	46
301	655	504	652
3465	7742	4888	5636
6665	21447	10724	13541
171	363	250	153
428	1097	535	557
52	224	97	94
74	444	105	219
242	1349	573	514
4724	4545	4870	3940
374	819	650	415
1107	3381	2017	2115
2	2	5	8
1	8	1	13
1	9	2	14
1	8	1	13
2	10	3	15
1	8	1	13
2	10	3	15
1	8	1	13
2	10	3	15
24204	30216	27916	25592
6051	7554	6979	6398
1513	1888	1745	1600
378	472	436	400
95	118	109	100
24	30	27	25

abanti data_11B_11B.RCC	abanti data_12A_12A.RCC	abanti data_12B_12B.RCC
abanti data_11B_11B.RCC	abanti data_12A_12A.RCC	abanti data_12B_12B.RCC
5450	24	59
843	749	739
22466	118	178
13898	39	97
364	245	271
37	14	45
4	1	3
16004	7925	7024
36	25	18
13	2	1
7	5	3
59	27	43
994	547	471
6924	4495	4940
18225	13736	11454
206	189	175
553	464	628
134	89	83
260	250	290
631	766	437
4477	2701	5508
582	589	501
2817	2326	2206
11	2	1
3	6	2
4	7	3
3	6	2
5	8	4
3	6	2
5	8	4
3	6	2
5	8	4
25532	31072	31072
6383	7768	7768
1596	1942	1942
399	486	486
100	121	121
25	30	30

Table 4.2: SIMPER test between two groups (toxic and non-toxic)



Taxon	Av. dissim	Contrib. %	Cumulative %	Mean Toxic	Mean Nontoxic
sxtA1_2	14.67	39.51	39.51	17.6	0.216
sxtA4	7.189	19.36	58.87	8.72	0.0923
sxtA	4.478	12.06	70.93	5.35	0.0702
sxtG_1	3.839	10.34	81.27	10.7	9.11
sxtI_2	3.134	8.441	89.71	13.6	14.9
ETIF	1.774	4.777	94.49	5.34	4.53
sxtI_1	0.6667	1.796	96.29	5.77	5.55
ICDH	0.3858	1.039	97.33	2.3	2.57
DYN	0.2231	0.601	97.93	0.579	0.72
sxtH/T_4	0.1994	0.5372	98.46	0.676	0.564
CPH	0.1346	0.3627	98.83	0.169	0.3
sxtB_1	0.1139	0.3069	99.13	0.406	0.289
GTEF	0.07715	0.2078	99.34	0.634	0.624
sxtA1_1	0.07157	0.1928	99.53	0.893	0.879
sxtU_1	0.06882	0.1854	99.72	0.61	0.637
sxtI_3	0.03504	0.09437	99.81	0.228	0.207
sxtU_2	0.02706	0.07288	99.89	0.117	0.106
sxtB_2	0.01991	0.05364	99.94	0.0333	0.0322
sxtH/T_3	0.01466	0.03948	99.98	0.058	0.044
sxtG_2	0.00757	0.02039	100	0.0199	0.023